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## PREFATORY NOTE

The circumstances under which the Department of Scientific and Industrial Research took over, from the Conjoint Board of Scientific Societies, the investigations into adhesives are detailed in the Introduction to this Report. The work which had been carried out for the Adhesives Committee of the Board under the direction of Professor Schryver was continued at the Imperial College of Science and Technology, and the greater part of the fresh experimental results here described are the outcome of the work of Professor Schryver and his assistants. Dr. J. C. Kernot was responsible for the investigations into casein and phenol-formaldehyde condensation products described in Sections III and VI respectively of this Report.

While preparing their Report the Adhesives Research Committee learned that Dr. T. Slater Price, Director of the British Photographic Research Association, had, by request of his Association, drawn up a descriptive bibliography of gelatin. The Council of the Association kindly consented to the publication of the bibliography as an Appendix to this Report, and Dr. Slater Price has been good enough to revise and bring it up to date.

The second Appendix to the Report has, at the request of the Adhesives Research Committee, been drawn up by Professor J. W. McBain, a recently appointed member of the Committee; the Appendix explains the bearing of recent work relating to soap systems by Professor McBain and his collaborators upon the structure of the gelatin gel.

The Report and Appendices are published upon the recommendation of the Advisory Council, who wish to express their indebtedness to all who have taken part in the work

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## MELTING AND FREEZING (SETTING) POINTS OF GELATIN

In the case of an emulsoid colloid such as gelatin, the change from a firm solid (the gel) through various phases of viscous semi-mobility to true colloidal solution (the sol), and *vice versa*, is so gradual and uniform that the process seems to be a continuous one, and it becomes difficult to apply the terms melting point and freezing point in the way in which they are used in connection with crystalline substances. The heat effects which occur when gelatin sets or melts have not been investigated very thoroughly and the information given in the literature is contradictory. Frank (*Koll. Beihefte*, 1913, **4**, 195) studied the heating curves of gelatin gels, using a thermocouple arrangement, but was not able to observe any appreciable absorption of heat on melting. On the other hand, Coblenz (*Chem. Zeit.*, 1918, **42**, 533) states that as soon as the sol begins to gel there is an arrest in the temperature; there may even be a rise of  $0.1^{\circ}$ - $0.2^{\circ}$ . (Compare also: Winkelblech, *Zeitsch. angew. Chem.*, 1906, **19**, 1260.)

Notwithstanding the above-mentioned uncertainties, various simple methods have been devised by means of which the so-called setting and melting points can be determined within very narrow limits of temperature. Among the crudest of these methods are those which depend on observing either the temperature at which a disc of gelatin begins to lose its shape (van der Helde, *Inaug. Dissert. Munich*, 1897; Chercheffsky, *Chem. Zeit.*, 1901, **25**, 413; Sammet, *J. Ind. Eng. Chem.*, 1918, **10**, 595) or the temperature at which the meniscus of a gel, contained in a tube, begins to take on a slope when the tube is inclined (Kissling, *Chem. Zeit.*, 1900, **24**, 567; 1901, **25**, 264; Levites, *Kolloid Zeitschr.*, 1907, **2**, 161).

One of the simplest methods, which is said to give very satisfactory results, is that due to Pauli (Pascheles) (*Pflüger's Archiv*, 1898, **71**, 333). The gelatin sol is cooled down in a vessel similar to that used in the Beckmann freezing point method, and the highest temperature at which there is a resistance to the withdrawal of the partially immersed thermometer is taken as the freezing or setting point; the lowest temperature at which the thermometer can be freely withdrawn from the gelatin (on warming) is taken as the melting point. In connection with this method it must be remembered that the behaviour of a gelatin sol or gel depends on its previous thermal history, and consequently the temperatures at which the sols set or the gels melt will depend on the velocity with which the heating or cooling takes place. With slow cooling the sol will set at a higher temperature than with fast cooling, *vice versa*, the more slowly the gel is heated, the lower is the temperature at which it melts. Wo Ostwald (*Kleines Praktikum der Kolloidchemie*, 2nd Edit., p. 76) defines the normal rate of heating or cooling as an alteration of  $1^{\circ}$  in 10 minutes, but the most convenient rate seems to be  $1^{\circ}$  in 5 minutes.

The difficulty of determining the temperature at which the gel melts has led various investigators to make use of some kind of external indicator. Herold, for example, encloses a thin layer of gelatin between the thermometer bulb and a surrounding glass tube, and notes the temperature at which the latter begins to fall away from the thermometer (*Chem. Zeit.*, 1910, **34**, 203; 1911, **35**, 93). Traube and Köhler (*Internat. phys.-chem. Biol.*, 1915, **2**, 42) make use of a method described by Schryver for cholate gels (*Proc. Roy. Soc.*, 1910, **83**, 96; 1914, **87**, 366). A test tube containing the gelatin sol, which has previously been warmed at  $65^{\circ}$ , is put into melting ice. The setting point is that point at which small glass

beads, which are strewed on the surface from time to time, only sink to half the depth of the gelatin.\*

Coblentz (*Chem. Zeit.*, 1918, **42**, 533) imprisons some small air bubbles in a sol contained in a glass cylinder, through the cork of which the thermometer passes down into the gelatin. The cylinder and contents are heated at 40°-50° and then put in a place at a temperature 2°-3° lower than the expected setting temperature, and shaken with a rotary motion from time to time. As soon as the solution begins to gel there is an arrest in the temperature for a short time; there may even be a rise of 0.1°-0.2°. At a slightly lower temperature, which is sharply defined, the air bubbles no longer follow the movement of the liquid.

Gamble (*Brit. Jour. Phot.*, 1910, **57**, 668) describes a method which is essentially a modification of the ordinary melting point method with capillary tubes, as used in organic chemistry. The capillary tubes, about 60 mm. long by 3 mm. in diameter, are attached to a thermometer dipping into a test tube of water which further dips into a large beaker of water. The water in the beaker is heated very slowly, and the melting point is taken as that temperature at which the concave meniscus gives place to a flat surface. The indication can be made more certain by placing a small shot on the gel in the capillary tube, at the moment the meniscus of the gel alters the shot sinks down through the gelatin (*private communication*). Full details are given of the method of preparing the gels and Gamble states that the individual determinations agree within 0.3°, but that with gels containing a high proportion of gelatin it is more difficult to obtain strictly concordant results.

Sheppard and Sweet (*J. Ind. Eng. Chem.*, 1921, **13**, 423) use a method the apparatus for which is based on that used by Flemming (*Zeitschr. physikal. Chemie*, 1902, **41**, 427) for the study of the rate of coagulation of colloidal silicic acids. The principle of the method is as follows: An intermittent stream of air bubbles, under constant pressure, is passed through the gelatin sol, the latter being cooled by ice water. A thermometer is immersed with its bulb near the orifice from which the bubbles emerge, and the temperature at which they cease to pass is taken as the setting point. *Vice versa*, after sufficient under-cooling, the set gel is surrounded with water at a definite higher temperature, and the melting point taken as the temperature at which the air bubbles again pass through. By an appropriate apparatus it is so arranged that the air bubbles pass every fifteen seconds under a slight but definite head of pressure. The orifice from which the bubbles issue is of definite shape and always kept at a fixed depth below the surface of the solution. The time necessary for the setting to take place with the different sols can also be registered. The authors also describe a simpler apparatus, which, however, is not so satisfactory.

#### *Setting and Melting Points of Pure Gelatin Sol and Gels*

In the sections on viscosity and swelling it was shown that the previous history of a gelatin sol has a pronounced effect on the results obtained, owing, in all probability, to changes which take place in the constitution of the sol. Arisz's experiments on the Tyndall effect indicate that similar changes take place in the gel. It follows, therefore, that the setting and melting points of sols and gels will also depend on the previous history of the systems, that is, in order to obtain comparable results the experiments should always be carried out under the same conditions. Neglect of such precautions has led to contradictory statements being

\* Actually, the time necessary for the beads to fall from the surface of the liquid to the bottom of the tube is also measured at definite intervals, and from the results the moment and temperature of setting is readily ascertained.

made in the literature. For example, Pauli and Roma (*Beitr. Chem. Physiol. Path.*, 1902, **2**, 1) state that the curve connecting the melting points of gelatin gels with their concentration is concave to the concentration axis, whilst the corresponding setting point curves are practically straight lines. Herold (*Chem. Zeit.*, 1910, **43**, 203) states that the relation between the concentration and the melting point is a straight line, and since the melting point will depend on the concentration of  $\alpha$ -gelatin, as distinguished from  $\beta$ -gelatin, the slope of this straight line is used to evaluate the quality of the gelatin, the slope being all the greater the better the grade of gelatin.

The effect of previous history can be predicted by analogy from the behaviour of gelatin with respect to viscosity and swelling. The following may, however, form a convenient summary of the behaviour of gelatin under given conditions (Wo. Ostwald, *Kleines Praktikum der Kolloidchemie*, 2nd Edit., p. 78). A 12 per cent. gelatin solution is divided into two parts. One part is kept in an ice chest, whilst the other part is preserved in a hot air oven in a conical flask fitted with a cork and capillary tube (to avoid evaporation). The two solutions are brought quickly to 40°, whereby the gel from the ice chest melts, and then the times necessary for gelatinisation to take place, the setting points and the melting points, are determined. The solution which was kept hot takes a longer time to gel and has a lower melting point than the solution which was kept in an ice chest.

The following results given by Gamble (*Brit. Jour. Phot.*, 1910, **57**, 668) bear on this point:—

A 5 per cent. gel after keeping for 4 hours at ordinary temperature gave the following melting points: 30.2°, 30.2°, 29.7°, 29.4°, 29.4°, 29.6°, 29.7°; mean 29.74°. A portion of the same gel was remelted and fresh tubes filled, which were then allowed to stand for 17 hours. The melting points were then: 30.8°, 30.6°, 30.8°, 30.8°, 31°, 30.8°, 30.8°, 31°; mean 30.82°.

A 10 per cent. gel, after standing in the tubes for 5 hours gave. 30.4°, 30.4°, 30.2°, 30.4°, 30.4°, 30.4°, 30.2°; mean 30.35°. After standing for 17 hours the melting points were: 30.8°, 30.6°, 30.8°, 30.8°, 31°, 30.8°, 30.8°, 31°; mean 30.82°.

As the concentration increases less differences in the melting points are found between tubes which have stood for shorter and longer periods. Generally there are greater variations between individual measurements when the tubes have stood for a short period.

Herold (*Chem. Zeit.*, 1910, **34**, 203) allowed a 10 per cent. solution to set for 12 minutes at different temperatures, and then determined the melting point. The curve showing the relation between the melting point and the setting temperature gave a sharp maximum at 23°. With a 20 per cent. gel and a setting time of 10 minutes the maximum was not so pronounced. Such a maximum is evidently a result of two factors which affect the rate of attainment of equilibrium, namely, the temperature, and the distance the system is removed from the point of equilibrium.

The following table shows the relation between the concentration and the setting and melting points, as given in Ostwald's book (p. 78):—

|                        |     |       |       |       |        |       |       |       |
|------------------------|-----|-------|-------|-------|--------|-------|-------|-------|
| Percent. concentration | 12  | 8     | 6     | 4     | 3      | 2     | 1.5   |       |
| Setting point          | ... | 21.5° | 19.9° | 17.9° | 14°(?) | 12.5° | 6.9°  | 1.8°  |
| Melting point          | ... | 27.6° | 26.9° | 26.3° | 24.1°  | 21.4° | 19.0° | 15.4° |

The results are shown graphically in Fig. 12.

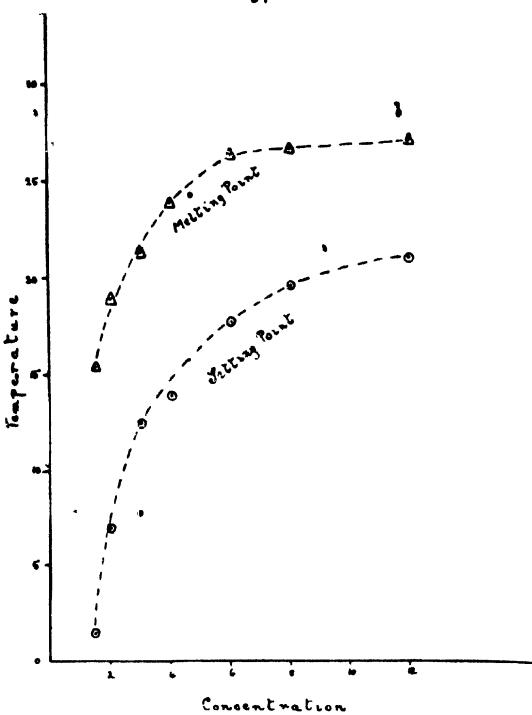


Fig. 12.

As distinct from the ordinary crystalline substances the melting point is much higher than the setting point, a fact which would appear to indicate that the gel is much more complex in its constitution than the sol.

Sheppard and Sweet (*J. Ind. Eng. Chem.*, 1921, **13**, 423) investigated 1, 3, 5, 10, 15, and 20 per cent. sols, the percentage being referred to gelatin dried at 105°. They were prepared under standard conditions, which were not defined more exactly than by saying that there was (1) a definite period of swelling in cold water, (2) a definite period of heating and stirring at 70°, (3) a definite short period of heating at 100°. 20 cc. of the solution thus prepared were placed in a 1 1/2 inch test tube, this fixed in a wider test tube (2 inch) serving as an air jacket and the whole immersed in the cooling vessel. The rate of cooling and heating are not given, but judging from the curves given in the illustration of the results (which are not given in detail), it is about 1° in 2-3 mins; apparently it is not the same in all cases.

The curves showing the relation between the concentration and the setting and melting points do not coincide, and there is an approximately constant difference in temperature between the setting and melting points at the different concentrations. The curves for different gelatins frequently cut each other.

#### Influence of Acids and Alkalies on Gelatinisation.

Loeb (*J. Gen. Physiol.*, 1921, **4**, 73, 97) has shown that the rate of increase of viscosity of a gelatin solution is greater at the isoelectric point than in more acid solutions. Since the time taken for gelatinisation, and hence the setting point, will probably be connected with the rate of increase in viscosity, it might be expected that at some low concentration of acid the setting point would be raised, that is, gelatinisation would

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be accelerated. At higher concentrations gelatinisation should be retarded, the setting point being lowered. The results given in the literature are contradictory, however, possibly because the experiments were carried out under different conditions. Traube and Köhler (*Internat. Zeit. phys.-chem. Biol.*, 1915, **2**, 42), who cooled the sol (1·75 per cent.) previously heated to 65°, in melting ice, found that if acids were present in a concentration of less than 0·01 mol. per litre, the rate of gel formation was increased, whereas above this concentration they exert an inhibiting action, which, with the exception of very weak acids, reaches a maximum when the solution contains 0·019 mol. per litre.\* The melting point of the gel (heated in a bath at 26°) is affected in exactly the reverse manner. The acids investigated were: Hydrochloric, nitric, sulphuric, *o*-phosphoric, phosphorous, hypophosphorous, boric, hydrocyanic, acetic, chloro-, dichloro- and trichloro-acetic, *i*-valeric, oxalic, succinic, tartaric, citric, and salicylic acids. Bases (potassium hydroxide, diethylamine, piperidine, pyridine) inhibit formation and accelerate solution of the gel.<sup>†</sup>

On the other hand, Fischer, (*Kolloid Zeitschr.*, 1915, **17**, 1) found that in the presence of 0·01 N-HCl the setting point of a 2 per cent. sol was lowered. Both hydrochloric acid and sodium hydroxide were found to have a very pronounced influence on the liquefaction of the gel, the influence becoming more marked as the concentration of the acid or alkali was increased. The presence of acid or alkali not only retards the process of gel formation, but also liquefies the gel already formed.

Coblentz (*Chem. Zeit.*, 1918, **42**, 533) states that acids and alkalis lower the setting point, equivalent quantities of citric, tartaric and glycolic acids having the same effect.

The addition of sodium chloride to a hydrochloric acid solution of gelatin should repress the dissociation of the gelatin hydrochloride present and therefore have an effect on the setting point. Traube and Köhler (*loc. cit.*) found that for dilute solutions (N/50-N/100) of acid the effect of the addition of salts is to counteract the liquefying tendency of the acid or base. A similar result is obtained by the addition of salts to sodium hydroxide gelatin. The salts used were: sodium chloride, calcium chloride, sodium sulphate, potassium chloride, potassium bromide, potassium iodide, potassium nitrate, potassium thiocyanate, potassium sulphate, potassium citrate and potassium tartrate, the last two being the most effective.

Fischer and Coffmann (*J. Amer. Chem. Soc.*, 1918, **40**, 303) have investigated the effect on 1 per cent. gelatin at 20° of solutions varying in composition from ortho-phosphoric acid to sodium hydroxide, through the intermediate mixtures with sodium dihydrogen phosphate and disodium hydrogen phosphate; similarly with citric acid in place of phosphoric acid. These solutions were therefore similar to those used by Fischer and Hooker (*J. Amer. Chem. Soc.*, 1918, **40**, 272) in their experiments on swelling. The gelatin remains set in the middle solutions of the series, that is, in the neighbourhood of the isoelectric point, there being a progressive increase in fluidity on either side as the concentration of the hydron or the hydroxide ion increases. With solutions varying in composition between sodium hydroxide and sodium carbonate, the gelatin remains set at a composition corresponding with sodium hydrogen carbonate (the swelling is also at a minimum at this point), but tends to liquefy as the bicarbonate is replaced by carbonate or by pure sodium hydroxide. Patten and Johnson (*J. Biol. Chem.*, 1918, **38**, 179) have carried out similar experiments with a 3 per cent. solution at 21°, at the same time measuring the pH of the various solutions. They

\* Traube and Köhler state definitely that the concentration of acid which produces the maximum inhibiting action on gel formation is approximately that which has the greatest effect on swelling.

<sup>†</sup> Compare, however, Davies, Oakes and Brown (*J. Amer. Chem. Soc.*, 1921, **43**, 1526) who state that the rate of increase in viscosity of a 1 per cent. solution is a maximum at pH = 8·8.

find that a 3 per cent. gelatin liquefies between pH = 8·4 and 9·2 in sodium bicarbonate solutions; between pH = 8·4 and 10·1 in 0·01-N phosphoric acid solutions; and between 8·4 and 9·6 for citric acid solutions. These data make it clear that the setting of gelatin is influenced by the hydrogen ion concentration of the medium. The following figure (13) shows the effect of temperature on the setting of 3 per cent. gelatin in 0·1-N phosphoric acid-sodium hydroxide solutions from 18°-25°; the narrowing of the pH range for solid gelatin solutions due to the rise in temperature is clearly seen.

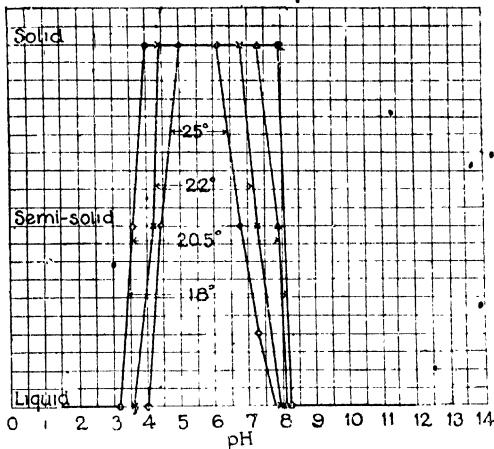


Fig. 13.

Fischer makes use of the results he has obtained with his various collaborators to support his theory that the hydration and the solution of a protein are not one and the same thing.

#### *Influence of Salts on the Gelatinisation.*

In connection with the influence of salts on the setting and melting points of gelatin a mass of data has been published which is difficult to summarise, owing to the fact that the concentrations of the salt to summarise, owing to the fact that the concentrations of the salt solutions and of the gelatin solutions are very different in the several papers. In no case has the pH of the various solutions been measured, so that it is not possible to use that as a guide to the results. The most careful work seems to have been done by Pauli and his co-workers, and by Traube and Kohler, and their results will be dealt with first.

Pauli (Pascheles) (*Pflüger's Archiv*, 1898, **71**, 333) and Pauli and Roma (*Beitr. Chem. Physiol. Path.*, 1902, **2**, 1) used solutions which were 10 per cent. with respect to gelatin, whilst the concentrations of the various salts varied up to 5 normal, unless coagulation of the gelatin first took place. They found that the chlorides, bromides and iodides of sodium, potassium and ammonium lower both the melting point and the setting point of 10 per cent. gelatin. Generally speaking, the lowering is not very marked at first, but as the concentration of the added salt is increased the effect becomes more and more pronounced. On the other hand the sulphates of sodium, potassium, magnesium and ammonium all raise the setting point and melting point. Generally there is a parallelism between the curves showing the relation between the concentration of salt and the setting and melting points; also the variation in the percentage of gelatin leaves the relative influence of the various salts unaltered.

Comparison of the results obtained with a large number of different salts shows that the anion has the preponderating effect, that of the cation being only subsidiary.\* In the order of favouring gel formation the anions can be arranged thus:—



Salts on the left side of  $\text{H}_2\text{O}$  raise, whilst those on the right side lower, the setting and melting points.

The effect of the cations is shown by the order



The action of the various salts does not seem to depend on their ionization or on their power of precipitating gelatin. For example, sodium chloride and ammonium chloride have very nearly the same effect on the setting point but ammonium chloride does not precipitate gelatin at any concentration, in contradistinction to sodium chloride. Sodium acetate and sodium chloride both precipitate gelatin, but have opposite effects on the setting point.

Experiments with mixtures of salts (in equivalent concentration) showed that the united effect of a mixture is the algebraical sum of the individual effects. It is thus possible to prepare a mixture of salts which has no action on the setting point or melting point of gelatin.

Traube and Köhler (*Internat. Zeit. phys. Chem. Biol.*, 1915, **2**, 42) used a 1·75 per cent. solution of gelatin, whilst the concentrations of the salt solutions varied only up to 0·045 equivs. per litre. For potassium, sodium and calcium salts the order of the anions with respect to the acceleration of the formation of, and inhibition of the solution, of a gel is:—

trichloroacetate > salicylate > iodide > bromide, and cyanide > nitrate > chloride > sulphate > citrate, whilst the order of cations is:—



Above a gelatin concentration of 2·7 per cent., however, the anion order is reversed, both with respect to the effect on the formation and solution of the gel, that is, the order is the same as that obtained by Pauli. 2·7 per cent. is the limiting concentration, or reversal concentration for KI; it was not determined for other salts.

Levites (*J. Russ. physiko-chem. Ges.*, 1901, **33**, 726; 1902, **34**, 110, 441; 1903, **35**, 935, 1004; 1904, **36**, 401; *Kolloid Zeitschr.*, 1907, **2**, 161. See also Daste and Floresco, *Archives de physiol.*, 1895, Morner, *Zeitschr. f. physiolog. Chem.*, 1899) has carried out experiments with some 80 different salts, but his results do not add much to what has already been stated. Salts of monobasic acids are said to retard, whilst those of di- and poly-basic acids accelerate the formation of a gel; there are exceptions, however, since alkali formates and acetates accelerate gelatinisation. Levites puts forward the proposition that the more readily gelatin dissolves in a solution the less rapidly will the sol gelatinise; e.g., gelatin does not dissolve in cold water, but it readily does so in a 15 per cent. solution of potassium iodide, and the sol formed gels only very slowly.

Steele (*Zeitschr. physikal. Chemie*, 1902, **14**, 713) gives a number of facts with respect to the action of various salts on 12 per cent. gelatin solutions, and Larguier des Baucels (*Comptes rendus*, 1908, **146**, 290) describes the solvent action of twice-molar solutions of various salts on gelatin, but no new facts are adduced. Coblenz (*Chem. Zeit.*, 1918, **42**, 533) states that alums raise the setting point, and Briggs and Hieber (*Jour. Physikal. Chem.*, 1920, **24**, 74) obtain the usual series with a 5 per cent. gelatin solution and either 33 per cent. or saturated solutions of

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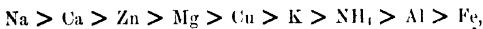
\* This statement is easy to understand if the gelatin is on the acid side of the isoelectric point, but unfortunately the only detail given about the gelatin is that it was salt-free.

various salts. The latter, as also does Baucels, showed that the various salts can be removed from the gelatin solutions by dialysis, the original properties of the gelatin being regained. Wo. Ostwald (*Kleines Praktikum der Kolloidchemie*, 1921, pp. 79-80), using 3 per cent. gelatin solution and semi-normal solutions of various potassium salts, gives the following order of anions with respect to their influence on the time of gelatinisation at the ordinary temperature, the solutions having previously been heated to 40° for 10-12 minutes.

Sulphate > citrate > oxalate > acetate > chlorate > carbonate > nitrate > bromide > cyanide > thiocyanate > iodide > salicylate.

The salts as far as the carbonates accelerate the rate of formation of the gel, whilst the remaining salts have a retarding action, as compared with the pure aqueous solution of the gelatin. Cyanides, thiocyanates, iodides and salicylates, at the concentration given and any higher concentration, completely prevent gel formation. This anion series is said to hold for slightly acid as well as slightly alkaline gelatins.

- The influence of cations, with sulphate as the anion, is practically negligible; there does not appear to be any sulphate, which, at the given concentration, will retard the rate of gelatinisation. Generally the cation series varies with the concentration of the salt, as also with the acid or alkaline reaction of the gelatin.\* For example, for a weak acid gelatin the order was



whilst for weakly alkaline gelatin the order was



the salts with the exception of  $\text{CaSO}_4$ , all being in 0.5 N. solution

#### *Influence of Non-electrolytes on Gelatinisation.*

The general results obtained may be stated as follows --

##### *Substances which lower the setting point or retard gelatinisation.*

Monohydric alcohols,  $\text{C}_n\text{H}_{2n+1}\text{OH}$ , the effect being the more marked the greater the molecular weight of the alcohol, [Traube and Köhler (*loc. cit.*) state, however, that methyl alcohol accelerates the rate of gel formation (1.75 per cent. gel) at concentrations of 0.8 and 1.0 molar, although it retards at 3-molar] ethyl ether, chloroform, chloral hydrate, ethyl acetate, urea, thiourea, furfural.

##### *Substances which raise the setting point or accelerate gelatinisation.*

Polyhydric alcohols, including glycerol and the sugars (sucrose, mannite, etc.).

The above results are due to Pauli, Levites, de Baucels, Traube and Köhler, Coblenz, and Briggs and Hieber. (*loc. cit.*)

\* This again points to the necessity of knowing the pH of the solutions.

## COAGULATION AND PRECIPITATION OF GELATIN

In an ordinary colloidal solution there is a uniform distribution of the disperse phase in the dispersion medium, the disperse phase being of submicroscopic dimensions. Under certain conditions the submicroscopic particles can be made to aggregate together so that they become of microscopic dimensions; accompanying this aggregation there is a loss of uniform distribution of the disperse phase in the dispersion medium and the sol is said to *precipitate*, *clot* or *coagulate*. This is quite different from gelation or gelatinisation, where the liquid sol changes as a whole into a solid gel; in the case of gelatin it is still doubtful whether the gel is homogeneous or heterogeneous.

Precipitation is generally brought about by the addition of an electrolyte to the sol, and in the case of suspensoid\* sols it is claimed that fairly simple relations hold. Suspensoid sols are either electronegative or electropositive in character, that is, the particles wander either to the anode or cathode on electrolysis. When an electrolyte is added to an electronegative sol, for example, the positive ion of the electrolyte will be adsorbed by the colloidal particles and tend to neutralise the negative charge on the latter; when the particles become electrically neutral, that is, isolectric, they coagulate and precipitation takes place (compare Hardy, *Journ. of Physiol.*, 1899, **24**, 288). Since the charge on an ion depends on its valency, it follows that a cation of higher valency should have a greater precipitating effect than one of lower valency. Schulze (*Journ. prakt. Chemie*, 1882, **25**, 431; 1883, **27**, 320) found that the relative precipitating powers (precipitating power = reciprocal of the concentration in gram-molecules per litre necessary to coagulate a given solution of the hydrosol) of the univalent, bivalent and trivalent metals, that is, of the cations, with respect to colloidal arsenic sulphide are in the ratio  $1 : 30 : 1650$ . Prost (*Bull. Acad. Roy. de Belg.*, 1887, [3], **14**, 312) and Linder and Picton (*Trans. Chem. Soc.*, 1895, **67**, 63) obtained similar results, whilst Whetham (*Phil. Mag.*, 1899, [5], **48**, 474) gave an interpretation, based on the theory of probability, of Schulze's valency rule. Later, Linder and Picton (*Trans. Chem. Soc.*, 1905, **87**, 1906) showed that for positive hydrosols (ferric hydroxide) the valency of the anion is the chief factor affecting the precipitation. These results, for both positive and negative hydrosols, were generally confirmed by Freundlich (*Zeitschr. physikal. Chemie*, 1910, **73**, 385), but the most recent work has shown that the so-called law of Schulze is only a first approximation and should be considered only as a guide. For example, Odén, in experiments on negative colloidal sulphur (*Der kolloide Schwefel*, 1912, p. 156) found that instead of univalent cations precipitating at the same concentration, the required concentration of lithium chloride is about 100 times that of caesium chloride. For different bivalent ions the precipitating powers range from 461 for barium to 13·2 for zinc, a ratio of over 30 : 1, instead of being the same. The univalent caesium ion has a greater precipitating power than the bivalent zinc, cadmium and nickel ions, and about the same precipitating power as the bivalent copper, manganese and magnesium ions, etc. The specific nature of the adsorption in the case of sulphur is thus demonstrated most clearly. With different colloids (platinum, silver, mastic, arsenic sulphide, ferric hydroxide, etc.), different orders of anions and cations with respect to their precipitating powers are obtained (compare Bancroft, *Applied Colloid Chemistry*, 1921, pp. 212 seq.), so that the results are not of such a simple nature as was first thought to be the case.

The above short account of the behaviour of suspensoids indicates the complicated nature of the phenomenon of precipitation or coagulation. With the protein emulsoids there is the further complication of their

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\* Wo. Ostwald calls the sols with a solid disperse phase in a liquid medium "Suspensoids," and those with a liquid disperse phase in a liquid medium, "Emulsoids."

amphoteric nature, so that their behaviour is likely to be more complex than that of the suspensoids. For example, the previous history of the emulsoids plays an important part; when albumin is precipitated by calcium chloride the coagulation is reversible if the calcium chloride is washed out at once, but it is not reversible if the precipitate is allowed to stand.

Gelatin is not so readily precipitated as other proteins, so that most of the work on precipitation\* has been done on such substances as albumin, egg-white, globulin, etc. Hofmeister and his students were the first systematically to investigate the precipitating action of salts on various proteins, including gelatin (*Archiv f. experiment. Pathol. und Pharmakol.*, 1888, **24**, 1, 247; 1888, **25**, 1; 1890, **27**, 395; 1891, **28**, 210, etc.) and the suggestion of Virchow (*Archiv path. Anat. und Physiol.*, 1854, **6**, 572) that the added salts render the protein insoluble by extracting water from them was again put forward. Using various sodium salts, Hofmeister found the following anion series for the precipitation of egg-white —

Citrate > tartrate > sulphate > acetate > chloride > nitrate > iodide > thiocyanate.

Pauli, however, in the case of gelatin found the following order for sodium salts (*Pflüger's Archiv*, 1898, **71**, 333; *Beitr. chem. Physiol. Path.*, 1902, **2**, 1). —

Sulphate > citrate > tartrate > acetate > chloride.

Moreover, Pauli pointed out that with the following salts the order when arranged with respect to their power of precipitating gelatin is slightly different from that when arranged with respect to their favourable action on gelatinisation.

#### *Precipitation.*

$\text{Na}_2\text{SO}_4 > \text{K}_2\text{SO}_4 > \text{Na citrate} > \text{MgCl}_2 > \text{Na tartrate} > \text{MgSO}_4 > (\text{NH}_4)_2\text{SO}_4 > \text{Na acetate} > \text{KCl} > \text{NaCl}$ .

#### *Gelatinisation.*

$\text{Na}_2\text{SO}_4 > \text{K}_2\text{SO}_4 > \text{Na citrate} > (\text{NH}_4)_2\text{SO}_4 > \text{MgSO}_4 > \text{Na tartrate} > \text{Na acetate}$ .

It was also pointed out that the influence of electrolytes on the precipitation of gelatin from its solutions is entirely distinct from the influence exerted by them on the setting and melting points. For example, salts which are able in precipitating gelatin may in the one case hinder gelatinisation (potassium chloride and sodium chloride), and in the other case accelerate it (sulphates, acetates and citrates).

Pauli also came to the conclusion that if a salt which by itself liquefies gelatin, lowers the dissociation of another salt dissolved in the gelatin, precipitation of the gelatin will be favoured. For example, with a warm 10 per cent. gelatin 4.25-N NaCl gives a slight cloudiness, the addition of N-NaBr (common Na<sup>+</sup> ion) gives immediately a very marked cloudiness (precipitation), whereas potassium bromide and ammonium bromide have no effect. It was also found that the addition of the non-electrolytes, urea, sucrose, and dextrose, diminished the precipitating power of the salts.

In these experiments the anion of the salts used seemed to have the preponderating influence. In further investigations, using egg-white, Pauli (*Beitr. chem. Physiol. Path.*, 1902, **3**, 225) found that a number of salts which will not coagulate egg-white by themselves will increase the

\* Attempts have been made to distinguish between precipitation and coagulation as follows (Brailsford Robertson, *The Physical Chemistry of the Proteins*, 1920, p. 107):— “Precipitation of a protein takes place through chemical interaction with the added salt, whilst coagulation is caused by a change in the nature of the solvent resulting from the further addition of salt.” At present, however, it is doubtful whether the phenomenon is sufficiently understood for such a distinction to be drawn.

coagulating power of other salts when mixed with them, while others markedly decrease the coagulating power of salts which, in their absence, readily coagulate egg-albumin. Moreover, certain salts, although very soluble, do not in any concentration cause coagulation of egg-white. The possibility suggested itself that the coagulating action of salts might depend on two antagonistic factors, respectively attributable to the cations and anions of the salts. In confirmation of this view it was found that the ammonium ion,  $\text{NH}_4^+$ , when combined with the sulphate ion,  $\text{SO}_4^{2-}$ , will coagulate egg-albumin, although when combined with the acetate ion it will not; acetate ion, however, coagulates when combined with a sodium ion,  $\text{Na}^+$ . Pursuing this line of reasoning and investigation, Pauli concluded that if the coagulating powers of a series of cations be indicated by  $f, f', f'', \dots$  and the opposite (solvent) powers of a series of anions by  $h, h', h'', \dots$  then in a mixture of electrolytes the following possibilities exist:—

$$\sum (f, f', f'', \dots) \geq \sum (h, h', h'', \dots),$$

the mixture being such as to coagulate, leave unaffected, or inhibit the coagulation of the albumin. Pauli found that in egg-white (in which the protein is electronegative) the cations of added electrolytes are the active agents in inducing coagulation, while the anions inhibit coagulation. In the following table of Pauli's the cations are arranged in ascending order of precipitating power from the left to right, while the anions are arranged vertically, the weakest inhibitor coming first and the strongest last. A "+" sign indicates that the salt which results from the union of the cation and anion causes coagulation of egg albumin, while a "-" sign indicates that it does not.

| Anions.        | Cations. |               |    |    |   | Li |
|----------------|----------|---------------|----|----|---|----|
|                | Mg.      | $\text{NH}_4$ | K  | Na |   |    |
| Fluoride ..    | ..       | ..            | .. | .. | + | +  |
| Sulphate ..    | ..       | ..            | .. | .. | + | +  |
| Phosphate ..   | ..       | ..            | .. | .. | + | +  |
| Citrate ..     | ..       | ..            | .. | .. | + | +  |
| Tartrate ..    | ..       | ..            | .. | .. | + | +  |
| Acetate ..     | ..       | ..            | .. | .. | - | +  |
| Chloride ..    | ..       | ..            | .. | .. | - | +  |
| Nitrate ..     | ..       | ..            | .. | .. | - | +  |
| Chlorate ..    | ..       | ..            | .. | .. | - | +  |
| Bromide ..     | ..       | ..            | .. | .. | - | -  |
| Iodide ..      | ..       | ..            | .. | .. | - | -  |
| Thiocyanate .. | ..       | ..            | .. | .. | - | +  |

It was, however, shown by Posternak (*Annales de l'Institut Pasteur*, 1901, **15**, 85) and later confirmed by Pauli (*Beitr. Chem. Physiol. Path.*, 1903, **5**, 27) and Hoeber (*ibid.*, 1904, **5**, 432) that the order of effectiveness of the different salts in bringing about the coagulation of electropositive protein is exactly the reverse of their order in bringing about the coagulation of electronegative protein, such as the albumin in egg-white (*compare* Froundlich, *Zeitschr. physikal. Chemie*, 1903, **44**, 129). Acidifying a solution of egg-white reverses the functions of the coagulating ions; those which coagulated electronegative protein most strongly now inhibit its coagulation most strongly. The series is reversed in every respect; the anions now induce coagulation and the cations inhibit it. The anions precipitate in the order:—



while the cations inhibit precipitation in the order:—



The above, although it does not deal specifically with gelatin, indicates how it gradually became recognised that the behaviour of a protein, which is amphoteric in character, depends on whether it is acting as an acid or as a base.

Generally speaking, the results which have been obtained for proteins such as serum and egg albumin and haemoglobin can be applied to gelatin, since these proteins are stable at the isoelectric point, but not necessarily the results obtained for globulin and casein, since these precipitate out when they are electrically neutral. A very large number of papers has been published on the proteins, but only gelatin will now be dealt with more specifically.

It has been mentioned that the influence of salts on the precipitability of proteins has been ascribed to the dehydrating effect of these salts. According to Pauli's theory the ions of proteins are strongly hydrated and therefore ionic protein is much better able to withstand dehydrating influences than amphoteric protein, and consequently the precipitation of protein by alcohol should either be diminished or quite done away with when the protein is ionised. Schorr (*Biochem. Zeitschr.*, 1911, **37**, 424) showed that the addition of hydrochloric acid to gelatin has a retarding effect on its precipitation by alcohol, but Loeb (*J. Gen. Physiol.*, 1920, **3**, 247) investigated the subject in greater detail. He found that when gelatin is treated with hydrochloric, oxalic, phosphoric, tartaric, succinic, citric, acetic, monochloroacetic, dichloroacetic or trichloroacetic acid, the following results were obtained. In carrying out the tests, 95 per cent. alcohol was added to 10 cc. of a one per cent. solution of gelatin in a test tube of definite diameter until certain letters became illegible when looked at through the test tube filled with the gelatin-alcohol mixture, the temperature being 10°. 10 cc. of isoelectric gelatin required 2 cc. of 95 per cent. alcohol to bring about the test opacity. When the pH was lowered to 4.55 by the addition of the above acids, 5.5 cc. of alcohol were required, whereas with pH = 4.50, 25 cc. or more of alcohol gave no precipitation but only a slight turbidity. With a pH of 4.45 or less the gelatin solution remained quite clear, no matter how much alcohol was added. With sulphuric acid, however, which gives a bivalent anion, the gelatin is readily precipitated at all pH values.

Similar results were obtained with gelatinates ( $\text{pH} > 4.7$ ) of the alkali and alkaline earth metals. Lithium, sodium, potassium and ammonium gelatinates (univalent cation) are no longer precipitable when the pH = 5.0 or more. The barium and calcium gelatinates (bivalent cation) are precipitated by small quantities of alcohol at any pH.

When the hydron concentration of a gelatin hydrochloride solution becomes very high its solubility in an alcohol-water mixture will again be diminished. This happens when the equivalent of 30-40 cc. of normal hydrochloric acid is contained in 100 cc. of a 2 per cent. solution of originally isoelectric gelatin.

In a previous paper, Loeb (*J. Biol. Chem.*, 1918, **34**, 489) had investigated the effect of various salts, in addition to acids and bases, on the precipitation of gelatin by alcohol, but no pH measurements were made.

Feim (*J. Biol. Chem.*, 1918, **33**, 279, 439; 1918, **34**, 142, 415) gives a detailed account of the precipitation of gelatin by alcohol in the presence of various salts and mixtures of salts, and deals especially with the so-called antagonistic effect of different salts. His results, at first sight, appear to be very complicated, but if the later work of Loeb and others is taken into account, they are readily explicable, so that they need not be considered further.

Moeller (*Kolloid Zeitschr.*, 1921, **28**, 281) has investigated coagulation phenomena in the presence of sodium chloride and hydrochloric acid and applies the results to the question of the structure of gelatin.

Very little seems to have been done on the effect of temperature on the precipitation of gelatin,\* but the following experiments from Wo. Ostwald's *Kleines Praktikum der Kolloidchemie* may be quoted:—

"To 50 c.c. of 0·5-1 per cent. gelatin solution is added sufficient saturated solution of ammonium sulphate just to give a slight cloudiness at room temperature; a few drops of water are then added to clear the sol. The mixture is divided into three parts, one of which is maintained at room temperature, the others being kept respectively in a warm place and in an ice chest. After 24 hours the solution kept warm is still clear, that at room temperature is slightly cloudy, whilst the one which was kept in the ice chest shows a very marked cloudiness."

According to Lumière and Seyewetz (*Bull. Soc. Chim.*, 1908, [IV], 3, 743) the following organic compounds precipitate gelatin:—phenol, resorcinol, orcinol, hydroquinone, pyrocatechol, gallotannic acid, pyrogallol, *p*-nitrophenol, chlorophenol, picric acid, monochlorhydroquinone (adurolo), R-acid (2-naphthol-3:6-disulphonic acid), G-acid (2-naphthol-6,8-disulphonic acid), S-acid (1:8-aminonaphthol-4-sulphonic acid). Tannin (gallotannic acid) is the only substance which gives a precipitate insoluble in water.

The effects of the salts of heavy metals have been studied by Pauli and Flecker (*Biochem. Zeitschr.*, 1912, 41, 461) and Kehoe (*J. Lab. Clin. Med.*, 1920, 5, 443), but these will be dealt with in a later section. Compare also. Levites, *Kolloid Zeitschr.*, 1911, 8, 4.

The following quotation, which deals with gelatin as a precipitating agent itself, may be given from Bancroft's *Applied Colloid Chemistry*, pp. 227, seq.

"Since a colloid flocculated by water may be charged positively or negatively, there is no reason why it should not precipitate another colloid under suitable conditions. We usually consider the colloids flocculated by water solely as protecting colloids, but this is clearly an inadequate view, as is shown by the experimental data. Years ago Schulze (*Pogg. Ann.*, 1866, 129, 369) pointed out that small quantities of gelatin solution were as effective as lime or alum in causing a rapid sedimentation of clay and that addition of minute quantities of gelatin to barium sulphate simplified the question of filtration and washing very much. He of course could give no adequate explanation of the phenomenon; the real explanation was given by Billitzer (*Zeitschr. physikal. Chemie*, 1905, 51, 145) who called attention to the fact that while gelatin, agar-agar, etc. ordinarily checks the precipitation of colloidal solutions by electrolytes, small amounts of these substances have a precipitating action. This can be detected even when the gelatin produces no precipitation itself. Billitzer cites the experiments of Neisser and Friedemann in which it was found that a mastic emulsion containing a trace of gelatin is precipitated more readily by sodium chloride than when no gelatin is present. Billitzer found that gelatin precipitates such negative colloids as antimony sulphide and arsenic sulphide in acid or neutral solution, but does not precipitate positively charged sols such as hydrous ferric oxides.† Gelatin in ammoniacal solution precipitates hydrous ferric oxide though no precipitation occurs if ammonia is added to a mixture of gelatin and ferric oxide. Bismarck Brown, which is a positive colloid, is precipitated by an alkaline gelatin solution, while eosin is precipitated by an acidified gelatin solution.

Graham (*Trans. Chem. Soc.*, 1862, 15, 246) states that colloidal silica is precipitated by gelatin, the composition of the precipitate varying between 56 and 92 parts of gelatin per 100 of silicic acid."

\* See the last section of this report for the effect of the salts of heavy metals, where some temperature effects are given.

† This question has been studied more in detail by Pauli and Flecker (*Biochem. Zeitschr.*, 1912, 41, 461) and will be referred to in the next section.

## ACTION OF SALTS OF HEAVY METALS ON GELATIN. TANNING AND HARDENING

As will be seen later, the action of the salts of the heavy metals in hardening gelatin is probably connected with the formation of the colloidal hydroxides of the metals by hydrolysis. Before considering the salts, therefore, it will be best to deal with the behaviour of gelatin towards the colloidal hydroxides themselves.

At the end of the section on "Precipitation and Coagulation" a quotation was given from Bancroft's *Applied Colloid Chemistry* to the effect that "Bilzitzer (*Zeitschr. physikal. Chem.*, 1905, **51**, 145) finds that gelatin precipitates such negative colloids as antimony sulphide and arsenic sulphide in acid or neutral solution, but it does not precipitate positively charged sols, such as hydrous ferric oxide." The later work of Pauli and Flecker (*Biochem. Zeitschr.*, 1912, **41**, 461), which was carried out with purified ox-serum albumin (dialysed against distilled water for 8 weeks) and gelatin (dialysed for 30 days) has shown this statement must be modified. It was found that with serum albumin purified colloidal ferric hydroxide (or chromium hydroxide) gives a domain of optimum precipitation (1 ferric hydroxide : 3 albumin, by weight). With excess of ferric hydroxide there is no precipitation, while with increasing ratio of albumin to the hydroxide there is a less marked precipitation corresponding with the lesser content of hydroxide. The effect of added salts, alkalis and acids, is summarised in the following table, where the various zones have the following significations:—

Zone I. — Optimum zone of precipitation.

Zone II. — Inorganic colloid in excess, when compared with the optimum zone.

Zone III. — Albumin in excess when compared with the optimum zone.

+++ — Strong precipitation.

++— Weak precipitation.

--- No precipitation.

|          | Normality. | Zone I. | Zone II. | Zone III. |
|----------|------------|---------|----------|-----------|
| ·01 KCl  | ...        | +++     | +++      | ++ to --- |
| ·1 KCl   | .          | +++     | +++      | ---       |
| ·001 HCl | .          | +++     | ---      | ---       |
| ·05 HCl  | .          | --      | ---      | ---       |
| ·01 NaOH | .          | +++     | +++      | ---       |
| ·1 Urea  | ...        | +++     | ---      | ++        |

In the absence of albumin the colloidal ferric hydroxide used was just precipitated by 0·125-N KCl or 0·0007-N NaOH (threshold values).

It will be seen from the above that precipitation, which involves both the inorganic and organic colloid, depends on the relative proportions of the two colloids and also on the electrolytes which are present.

Gelatin gave similar results to albumin, but there was the difference that in all cases the gelatin was more sensitive to acids, very weak concentrations of hydrochloric acid being sufficient to prevent precipitation. An extract from the results is given in the following tables.

*Gelatin and Colloidal Ferric Hydroxide.*

Threshold values for the precipitation of ferric hydroxide in the absence of gelatin: 0·0125-N KCl; 0·007-N NaOH.

| %Fe(OH) <sub>3</sub> | % Gelatin. | No addition. | +0·01-N<br>KCl | +0·001-N<br>HCl | +0·01-N<br>NaOH | +0·1-N<br>Urea |
|----------------------|------------|--------------|----------------|-----------------|-----------------|----------------|
| 0·331                | .050       | ---          | +++            | ---             | +++             | ---            |
| .331                 | .062       | ---          | +++            | ---             | +++             | ---            |
| .331                 | .083       | ---          | +++            | ---             | +++             | ---            |
| .331                 | .125       | ---          | +++            | ---             | +++             | ---            |
| .331                 | .166       | ---          | +++            | ---             | +++             | ---            |
| .331                 | .250       | ++           | +++            | ---             | +++             | ++             |
| .331                 | .500       | +++          | +++            | ---             | +++             | +++            |
| 0·220                | .500       | +++          | +++            | ---             | +++             | +++            |
| .132                 | .500       | ++           | ++             | ---             | ++              | ++             |
| .094                 | .500       | ++           | ++             | ---             | ++              | ++             |
| .073                 | .500       | ++           | ++             | ---             | ++              | ++             |
| .060                 | .500       | ++           | ++             | ---             | ++              | ++             |

The column headed "No addition" shows the three zones previously mentioned, namely Zone II., where there is no precipitation and the ratio of Fe(OH)<sub>3</sub> to gelatin is in excess of that in Zone I., where there is optimum precipitation; as the ratio of gelatin to Fe(OH)<sub>3</sub> increases above that of the optimum zone, Zone III. is entered into, where the precipitation is much less.

Such results may appear to be confusing at first sight, but they are explicable when the following is borne in mind. Ferric hydroxide is a positive colloid (wanders to the cathode), whilst gelatin purified by dialysis will be on the alkaline side of the isoelectric point and will therefore act as a negative colloid. Since colloids tend to precipitate when they are electrically neutral there will be certain mixtures of ferric hydroxide and the gelatin where precipitation will rapidly take place owing to the approximate neutralisation of their respective charges; this will correspond with Zone I. When either colloid is in sufficient excess, the absorption complexes formed between the two colloids will be sufficiently removed from the isoelectric point for the mixture either to be stable or to give only a slight precipitate.

The explanation of the results obtained when electrolytes were added, is also now obvious, although it was not so when Pauli and Flecker made their experiments.

+ 0·01-N NaOH.—The addition of sodium hydroxide gives rise to the formation of sodium gelatinate, so that there will be a much greater concentration of negatively charged gelatin anions than in the case of pure gelatin. The charge on the ferric hydroxide will therefore be neutralised, even, when it is present in excess, and precipitation will take place in Zone II. as well as in Zone I. Zone III. will not be affected.

+ 0·001-N HCl.—The addition of the hydrochloric acid will cause the gelatin to be on the acid side of the isoelectric point, so that gelatin hydrochloride is formed, the gelatin ion being positive. There will thus be no neutralisation of the positive charge of the ferric hydroxide and precipitation will not take place in any of the zones.

+ 0·1-N Urea.—Urea is a non-electrolyte and will not affect the charge on the gelatin, so that the results will be the same as without the addition of urea.

+ 0·01-N KCl.—The action of potassium chloride is not so readily explicable, as it does not alter the pH of the gelatin. Since, however, when present in sufficient concentration it has a precipitating effect on

gelatin (see previous section) as well as on ferric hydroxide, it is possible that when added to the mixture of the two colloids this precipitating tendency is sufficient to transfer the mixture from Zone II, to Zone I.

The results with colloidal chromium hydroxide are similar in character to those with ferric hydroxide, but a little more complex.

#### Gelatin and Colloidal Chromium Hydroxide.

Threshold values for the precipitation of chromium hydroxide in the absence of Gelatin:—1-N KCl; 0.008-N NaOH.

| % Cr(OH) <sub>3</sub> | % Gelatin. | No addition. | +0.4 N<br>KCl | +0.001-N<br>HCl | +0.01-N<br>NaOH | +0.1-N<br>Urea |
|-----------------------|------------|--------------|---------------|-----------------|-----------------|----------------|
| 0.066                 | 0.050      | ---          | +++           | ---             | ---             | ---            |
| 0.066                 | .055       | ---          | +++           | ---             | ---             | ---            |
| 0.066                 | .071       | ---          | +++           | ---             | ---             | ---            |
| 0.066                 | .100       | ---          | +++           | ---             | +               | ---            |
| 0.066                 | .166       | ---          | +++           | ---             | +               | ---            |
| 0.066                 | .250       | ---          | +++           | ---             | ++              | ---            |
| 0.066                 | .500       | ---          | +++           | ---             | ++              | ---            |
| 0.033                 | .500       | ---          | ±±            | ---             | ++              | ---            |
| 0.022                 | .500       | ---          | +             | ---             | ++              | ---            |
| 0.016                 | .500       | ---          | ±             | ---             | ++              | ---            |
| 0.013                 | .500       | ---          | ---           | ---             | ++              | ---            |
| 0.011                 | .500       | ++           | ---           | ---             | +++             | ++             |
| 0.009                 | .500       | ++           | ---           | ---             | +++             | +++            |
| 0.008                 | .500       | ++           | ---           | ---             | ++              | +++            |
| 0.007                 | .500       | ++           | ---           | ---             | ++              | ++             |
| 0.006                 | .500       | ++           | ---           | ---             | ++              | ++             |

++ = strong cloudiness, but no precipitation.

± = weak cloudiness, but no precipitation.

The positive charge on chromium hydroxide is evidently greater than on ferric hydroxide, since the optimum zone of precipitation is only obtained when the ratio of gelatin to chromium hydroxide is much greater than with ferric hydroxide. If this is taken into account it will be seen that the results with added salts are all explicable in the same way as are those with ferric hydroxide.

Pauli and Flecker also investigated the precipitation of proteins by salts of the heavy metals, *e.g.*, by ferric chloride. This phenomenon gives rise to what are often known as "irregular series." If the protein is mixed with the salt in low concentration a precipitate is formed (Zone A). With higher concentrations of the salt the mixture remains clear over a certain zone, with re-formation of a precipitate (Zone B) when the concentration of the salt reaches still higher values which may be several times normal.

The precipitation in Zone A is explicable on the assumption that the salt undergoes hydrolysis with the formation of colloidal metal hydroxide,  $\text{Fe}(\text{OH})_3$ , if the salt is  $\text{FeCl}_3$ , which then gives a precipitate with the protein, in the way dealt with above. Evidence in support of this is the fact that if the precipitate is dialysed, chloride ions are found in the external liquid, but no ferric ions (using  $\text{FeCl}_3$ ).

Solution in the middle zone is due (according to Pauli and Flecker) to the formation of complexes between the protein and the salt, which complexes are dissociated and therefore hydrated and soluble. With excess of salt the dissociation of these complexes is suppressed; they are therefore dehydrated and precipitated (Zone B). Evidence in support of the formation of such complexes was obtained by the viscosity measurements, precipitation with alcohol, conductivity, and electrophoresis (albumin was used and not gelatin).

An alternative explanation of the middle zone and of zone B may, however, be given. When the salt undergoes hydrolysis, acid is formed as well as the colloidal hydroxide, and in medium concentrations of the

salt there will be sufficient acid liberated to transform the protein to the acid side of the isoelectric point; that is, the protein and the colloidal metal hydroxide will both be positively charged, and precipitation will not take place. This acid will also affect the swelling of the protein, etc., so that the viscosity and other properties of the solutions will be affected, as found by Pauli and Flecker. With more concentrated solutions of the salt the hydrolysis is not so marked\* (hydrolysis increases with the dilution) and precipitation effects may again come into play, either as in Zone A, or by the excess of salt decreasing the dissociation of the metal proteinate formed.

Gelatin differs from albumin in that it is much more sensitive towards acids, which even in small concentrations exert a marked protective action against precipitation. The tables already given show that very low concentrations of acid (0.001-N) prevent the precipitation with metal hydroxide, whereas with albumin precipitation is not prevented; e.g., 0.220 per cent.  $\text{Fe(OH)}_2 + 1.174$  per cent. albumin gives a precipitate in the presence of 0.001-N HCl. This difference is of importance in connection with the known different behaviour of gelatin and albumin towards the salts of heavy metals.

Reference has already been made to the hydrolysis of salts of heavy metals, and to the effect of dilution on the hydrolysis. The increased hydrolysis on dilution may readily be shown by the following experiments of Lippmann-Cramer (*Kolloidechemie und Photographic*, 1st Edit., p. 124). A solution of ferric chloride is diluted in the one case with water and in the other case with a 1 per cent. solution of gelatin. The latter solution will give the same dark colour as the aqueous solution does on boiling, showing the increased hydrolysis (hydrolysis increases with rise in temperature). Strictly speaking, however, this is not merely the effect of dilution. The colloidal ferric oxide is adsorbed by the gelatin, forming a complex of some kind, and thus the equilibrium represented by the equation,



is disturbed and more hydrolysis takes place; there is therefore the double effect of the dilution and of the influence of gelatin.

The increased hydrolysis due to the gelatin is shown still better by means of ferric thiocyanate. If the blood-red solution made by mixing equal volumes of 10 per cent. solutions of iron alum and ammonium thiocyanate is added drop by drop to a 10 per cent. solution of gelatin, coagulation takes place with the formation of ruby-red membranes. After a few minutes the colour disappears, giving place to that of rusty brown ferric hydroxide.

Solutions of ferric, chromic, and aluminium salts will always be acid, owing to the hydrolysis, and this is the explanation of why Lumière and Seyewetz (*Bull. Soc. Chim.*, 1902 [iii], **27**, 1073) were not able to alter the acidity of a solution of potassium chrome alum after five recrystallisations, a result at which they appear to be very surprised. Also the statement of Namias (*Phot. Korr.*, 1902, **39**, 446) that chromium chloride,  $\text{CrCl}_3$ , has no tendency to give the hydroxide in solution cannot be correct.

Experiments on the hardening of gelatin by chromic, iron and aluminium salts were made in the first case by Namias (*loc. cit.*) and A. and L. Lumière and Seyewetz (*Bull. Soc. Chim.*, 1902, [iii], **27**, 1073; 1903, [iii], **29**, 1077; 1906, [iii], **35**, 676). If a suitable amount of chrome alum is added to a gelatin sol, or if sheets of gelatin are soaked long enough in a chrome alum solution, the gelatin becomes insoluble even in boiling water and does not putrefy on standing, as the original gelatin

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\* Goodwin (*Zeitschr. physikal. Chemie*, 1896, **21**, 1) found that there is practically no hydrolysis, at 25°, with ferric chloride solutions of concentrations greater than 0.6 molar.

would do. Lumière and Seyewetz found first that gelatin could be rendered insoluble by treatment with almost any salt of the formula  $\text{Cr}_2\text{X}_6$ : sulphate, sulphite, nitrate, chloride, fluoride, acetate, formate, citrate or lactate. Since it had been shown by Namias (*Compare, also, Stolze, Eder & Jahrb.*, 1880, 330) that the power of chrome alum to make gelatin insoluble was increased by the addition of alkali up to a point where precipitation of chromium hydroxide occurred, experiments were made with the so-called green basic sulphate of Recoura.\* Gelatin treated with a solution of this salt resisted repeated washing with boiling water better than gelatin which had been treated with a solution that was not basic. On the other hand, gelatin is not made insoluble by treatment with the so-called solution of chromium hydroxide in potassium hydroxide † If the proper amount of ammonia is added to the gelatin and then a chrome alum solution, the gelatin becomes insoluble in boiling water, but this is not the case if ammonia is present in excess. Working with 2·5, 5 and 10 per cent. solutions of gelatin, it was found that two grams of chrome alum is the minimum quantity which can be added to 100 grams of gelatin to give a precipitate resistant to boiling water. When a gelatin solution is 5 per cent. or less in strength it does not become insoluble immediately, but only after keeping for some time. The time of keeping is all the longer the less the concentration of the gelatin, and appears to be about 50 hours for 2·5 per cent. gelatin.

The maximum amount of chrome alum that can be absorbed by 100 grams of gelatin is about 21 grams, independent of the concentration of the gelatin solution. Excess of chrome alum above this quantity is given up by the solid gel to cold water.

Experiments with chromic sulphate, chloride, nitrate, and acetate showed that approximately the same quantity of chromium hydroxide was necessary to make the gelatin insoluble, irrespective of the nature of the acid. Since this implies that only chromium hydroxide counts, experiments were made to see what became of the acid radicle. Sheets of gelatin were immersed in a chromium sulphate solution of known strength and the solution analysed afterwards. The ratio of chromium to sulphate in the solution was practically the same before and after treatment with the gelatin, which meant apparently that the chromium sulphate was taken up as a whole. It could not be the chromium sulphate as such which made the gelatin insoluble, because the same result could be obtained when the chrome alum was added to an ammoniacal gelatin. When the gelatin containing chromium sulphate was washed repeatedly with boiling water, acid was extracted very slowly, but after some washings the gelatin swelled a little and was carried off in the wash water, leaving the residue relatively richer in chromium, the chromium oxide content running up to 5·4-4·6 grams per 100 gms. gelatin. When the gelatin was treated with dilute alkali, it was possible to remove the acid without causing any swelling or any solution of the gelatin. When the gelatin was treated in this way the maximum amount of chromium found in it was 3·3-3·5 gms.  $\text{Cr}_2\text{O}_3$  per 100 grams gelatin.

Since the same result is obtained with all the different salts it is obvious that it is the chromium hydroxide, formed by hydrolysis, which makes the gelatin insoluble (*compare* the experiments of Pauli and Flecker); the acid of the salt is adsorbed strongly either by the chrome oxide, the gelatin, or both, most probably by the gelatin because of its amphoteric nature. When the acid is washed out at high temperatures, it causes a swelling of the gelatin. In fact, an insoluble gelatin, which has been freed from acid, swells rapidly and finally dissolves if diluted with water containing small quantities of acid or alkali.

\* This is really a sulphate of a complex radicle containing chromium and chlorine and has the composition  $[\text{Cl}(\text{Cr}(\text{H}_2\text{O})_6)]\text{SO}_4$ .

† This is probably due to the excess of alkali, which not only has a swelling effect on the gelatin but also peptises the chromium hydroxide.

For further experiments on the action of chromium salts, see Burton and others, *J. Soc. Leather Trades' Chemists*, 1920, **4**, 205; 1921, **5**, 183, 187; 192; 1923, **6**, 6, 14.

Both Namas (*loc. cit.*) and Lumière and Seyewetz (*loc. cit.*) showed that aluminium salts harden gelatin, but not so readily as chromium salts, and the latter investigators, by similar experiments to those described for the chromium salts, proved that the effect was due to the aluminium hydroxide (formed by hydrolysis). When aluminium hydroxide is freshly precipitated in gelatin by mixing equivalent quantities of ammonia and the aluminium salt, Lumière and Seyewetz state that the setting point of the gelatin rises proportionately to the quantity of aluminium salt used until the equivalent of 0·64 gm. of alumina ( $\equiv$  6 gms. of potassium alum) per 100 gms. of gelatin has been added. Further additions have at first no effect on the setting temperature, but finally cause it to fall. The maximum rise in the setting point is about 3·5° for 5 per cent. gelatin, 6° for 7·5 per cent. and 7° for 12 per cent., but the quantity of alum per 100 grams gelatin required to produce this maximum rise is the same in all cases, that is, it is independent of the concentration of the gelatin solution.

Abegg and von Schroeder (*Kolloid Zeitschr.*, 1907, **2**, 85) state that the melting point of a 10 per cent. gelatin is lowered from 36° to 34° and 32°, without visible tanning, by 10 per cent. solutions of potassium and chrome alum respectively; if the gelatin is first bathed in a solution of sodium carbonate before being put into the alum solutions, tanning takes place but the melting point is still lower than that of the original gelatin. Ferric alum, either with or without the soda bath, gives a hard brittle gelatin, m.p. 40°-38°.

Statements of this kind are hard to understand at first, until it is remembered that superposed on the tanning action, which gives a rise in the melting point, there is the ordinary salt effect, which lowers the melting point. For example, if the melting point of untanned gelatin is lowered from 36° to 33° in a given solution of sodium chloride, the melting point of tanned gelatin is lowered from 41° to 38°. Again, when gelatin is tanned with a salt and then washed, the melting point of the gelatin first rises, as the excess of salt and acid products of hydrolysis are washed out, and then falls as washing removes the tan. This is shown by the following results of Abegg and von Schroeder. The melting point of the untanned gelatin was 36°.

| After tanning with :   | To a m.p. of | m.p. after $\frac{1}{2}$ hour washing. | m.p. after long washing. |
|------------------------|--------------|--|--------------------------|
| 10% potassium alum ... | ...   ...    | 34°                                    | 42°                      |
| 10% chromium alum ...  | ...   ...    | 32°                                    | 37°                      |
| Iron Ammon. Alum ...   | ...   ...    | 48°                                    | 45°                      |
|                        |              |  | 41° (1 hour)             |

Abegg and Schroeder found that the viscosity of a dilute solution of gelatin (1·2 per cent.) is increased by the addition of potassium or chromium alum to an extent approximately equal to that which the same quantity of water experiences by the addition of these salts. Bogue (*J. Chem. Met. Eng.*, 1920, **23**, 5) found that the potassium alums of chromium, iron, and aluminium all increase the viscosity of gelatin, the chromium alum being the most effective.

Lüppo-Cramer (*Kolloidchemie und Photographie*, 1st Edit., pp. 121-152; *Kolloid Zeitschr.*, 1909, **4**, 21) has carried out numerous experiments on the tanning of gelatin by salts, and also comes to the conclusion that the effect is due to the hydroxides formed by hydrolysis. That the higher hydroxides of many metals have the faculty of tanning, whilst the lower

hydroxides of the same metals produce no such effects, is due to the fact that salts corresponding with the higher hydroxides are more generally hydrolysed, and the hydroxides are very stable in the hydrosol form.

Some of Lüppo-Cramer's experiments may be referred to a little more in detail:—In order to avoid disturbing coagulation effects, plates coated with gelatin are used instead of gelatin solutions. If the gelatin-coated plates are bathed in a two per cent solution of ferric chloride or of iron alum for 5 minutes, and then washed in distilled water for 30-40 minutes, the brownish-yellow colour of the gelatin, due to the adsorbed iron salt, does not disappear, and after drying the gelatin is quite insoluble in boiling water. If, however, after treatment with the iron salt the plates are bathed for 10 minutes in a 10 per cent. solution of ammonia, sodium hydroxide or potassium hydroxide, the colour of the film becomes markedly darker and, after washing, all traces of tanning have disappeared. It follows from this that insoluble ferric hydroxide, formed in the gelatin film by excess of alkali, does not give rise to tanning.

The different treatment also gives rise to a different behaviour towards oxalates and citrates. If the two plates, after being freshly made and washed for 30 mins., are bathed for 5-10 minutes in a 10 per cent. solution of potassium oxalate or of potassium citrate, the one treated with alkali remains unchanged, whilst the other becomes colourless in a few minutes, and after washing for half a hour is completely free from iron. There is, however, a time effect in this connection; with plates bathed for five minutes in 2 per cent. ferric chloride solution, the longer the washing with water before treatment with oxalate or citrate solution, the less soluble do the iron salts become. At the same time it is noticed that the colour of the gelatin film becomes darker as the washing proceeds. The tanning of the gelatin is not affected by the long washing.

If to 100 cc. of a 10 per cent. gelatin solution at 50°, 1-2 cc. of a 10 per cent. solution of ferric chloride or of iron alum are added, coagulation takes place at the places of contact and it is impossible to obtain a homogeneous mixture. If, however, a large excess of iron salt is used in concentrated solution there is no coagulation or tanning. Thus 50 cc. of the 10 per cent. ferric chloride or iron alum solution can be mixed at 50° with 50 cc. of a 10 per cent. solution of gelatin without coagulation occurring. A dark brown solution is obtained, which, after setting and keeping for several days, will melt unchanged at 40°. This is only an example of the "irregular series" referred to under Pauli and Flecker's work.

Similar results to the above were obtained with chrome alum and ordinary alum. Chrome alum differs from the iron and aluminium compounds, however, in that a mixture of a 10 per cent. solution with an equal or double volume of 10 per cent. gelatin does not melt again after setting. Also after tanning with chrome alum the product is much more resistant towards oxalates and citrates than after tanning with the other alums.

Lüppo-Cramer also describes experiments in which tanning was obtained with salts of uranium and gold and cerium, and with potassium permanaganate, owing to the formation of colloidal hydroxides. Results obtained with such substances as potassium ferrioxalate, potassium ferro- and ferricyanide, copper chloride and sulphate, silver nitrate, mercuric chloride, lead nitrate, barium chloride, potassium hydroxide and ammonia, where tanning, if it takes place at all, is only of very subsidiary importance, are also described.

When the hydroxides of silver, mercury, copper, nickel, cobalt, or ferrous iron are produced in gelatin in such a way that there is no excess of alkali hydroxide, e.g., sodium hydroxide, but rather a slight excess of the heavy metal salt present, tanning occurs to a greater or lesser extent. The so-called silver peroxide, from a silver salt and potassium or ammonium persulphate, also tan<sup>s</sup> gelatin, whilst lead oxide or hydroxide has no effect.

Colloidal silver iodide and bromide are able to coagulate gelatin. If 10 cc. of 10 per cent. silver nitrate are added to 50 cc. of a 10 per cent. solution of gelatin (temperature not above 50°), and then 10 cc. of 10 per cent. potassium iodide solution added, the gelatin coagulates completely to a gel, which can be melted on raising the temperature. The same phenomena occurs when colloidal silver bromide is formed in the gelatin, but the temperature must not rise above 40°. The colloidal sulphides of silver, mercury, lead, copper, gold, tin, iron, nickel, cobalt, zinc and cadmium cause either coagulation or complete tanning of the gelatin.

Kehoe (*J. Lab. Clin. Med.*, 1920, **5**, 443) describes experiments which would indicate that the coagulation of gelatin is not an irreversible reaction, but may be reversed through the action of alkalis or of the neutral salts of the alkali or alkaline earth metals.

Lumière and Seyewetz (*Bull. Soc. Chim.*, 1908, [iv], **3**, 743) added to a 10 per cent. solution of gelatin aqueous solutions of salts chosen from carbonates, chlorides, bromides, iodides, sulphates, sulphites, thiosulphates, nitrates, nitrites, hypochlorites, chlorates, bromatos, iodates, cyanides, thiocyanates, phosphates, borates, arsenates, arsenites, silicates, chromates, molybdates, tungstates, vanadates, ferro-and ferricyanides. As far as possible 5 per cent. salt solutions were used. The gelatin was precipitated only by solutions of the following: phosphotungstic acid; a mixture of phosphomolybdic acid and ammonium molybdate; a mixture of phosphotungstic acid and sodium tungstate; chlorine water, bromine water, ferric salts (except tartrates, citrates and certain double salts), manganic, vanadic, ceric and uranic salts, chloroauric acid, chloroplatinic acid, mercuric salts and potassium permanganate. It is shown that the insoluble gelatin retains some of the salts, and from the above list the conclusion is drawn that the salts which precipitate gelatin in dilute solution are those which are capable of furnishing oxygen either directly or indirectly. Lumière and Seyewetz realise that the converse, that all salts capable of furnishing oxygen will precipitate gelatin is not true. In making such a statement as this they were probably led astray by the inclusion of chlorine and bromine water in the list, and did not realise that in most of the cases, at all events, the effect on the gelatin was due to the hydrolytic products of the salts. In a later part of the paper they do admit, however, that the rendering insoluble of the gelatin is not solely an oxidation phenomenon, since the gelatin fixes a certain amount of the oxide (hydroxide) of the salt, "probably in the colloidal form as shown by Lippo-Cramer."

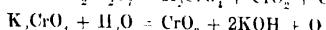
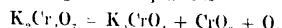
In a later paper Meunier and Seyewetz (*Bull. Soc. Chim.*, 1912, [iv], **11**, 344) show that sodium hypochlorite and hypobromite act similarly to chlorine and bromine water respectively in hardening gelatin, the action probably consisting in the formation of halogenated aminogroups in the protein molecule. (Compare Bevan and Briggs, *J. Soc. Chem. Ind.*, 1908, **27**, 260).

#### *Action of Salts of Chromic Acid on Gelatin.*

It has been stated that solid chromium trioxide,  $\text{CrO}_3$ , when preserved free from dust and in an evacuated, sealed tube, is stable towards light, although Lemoine (*Comptes Rendus*, 1881, **93**, 514; *Phot. Mitt.*, **18**, 244) maintains that after exposure to light for one and a half years it is decomposed with the evolution of oxygen. All chromates and dichromates, in pure aqueous solution, are stable to light (*Plotnikow, Zeitschr. wiss. Phot.*, 1919, **19**, 40; Matthews and Dewey, *J. Phys. Chem.*, 1913, **17**, 217); if, however, organic substances are present the action of light immediately causes a decomposition of the chromic acid salt, with the production, in many cases, of a brown, insoluble powder, and accompanied by the evolution of oxygen. The reactions which take place are probably very complicated; according to the composition of the organic substance used the chromates and dichromates may give coloured solutions containing trivalent chromium salts, but in most cases a dark brown precipitate

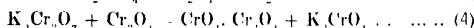
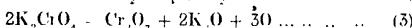
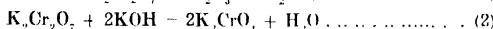
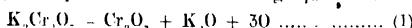
of variable and 'complex' composition is obtained. Even in the case of such simple substances as ethyl and methyl alcohols the statements made vary; Plotnikow (*Lehrbuch der Photochemie*, p. 413) states that a neutral solution a brown precipitate is formed, whereas Benrath (*Zeitschr. physikal. Chemie*, 1917, **16**, 253) claims that the product is a brilliantly green chromic hydroxide; in the presence of acetic acid a greenish-violet solution is obtained.

Since the reactions which take place with organic compounds of comparatively simple composition are so little understood (compare Goldborg, *Zeitschr. physikal. Chemie*, 1901, **41**, 1; *Zeitschr. wiss. Phot.*, 1906, **4**, 95; Luther and Forbes, *J. Amer. Chem. Soc.*, 1909, **31**, 770), it is no wonder that the reactions which take place with such a complicated substance as gelatin are by no means clear, although the property of gelatin, containing dichromate, of becoming insoluble on exposure to light, was discovered by Fox Talbot as far back as 1853. According to Eder (*Phot. Korr.*, 1878; *Handbuch d. Photogr.*, 1906, [1], **2**, 177; compare also Namias, *Phot. Korr.*, 1902, **39**, 446; Levites, *Kolloid Zeitschr.*, 1911, **9**, 1) potassium dichromate is reduced according to the equations —



a yellowish brown chromium dioxide having the composition  $\text{Cr}_2\text{O}_3 \cdot \text{CrO}_3$  ( $\equiv 3\text{CrO}_2$ ), that is, a chrome chromate, being produced. This compound is very unstable and gradually loses oxygen with the formation of green chromic oxide,  $\text{Cr}_2\text{O}_4$ , [or the hydrated oxide,  $\text{Cr}(\text{OH})_3$ ].

Lumière and Seyewetz (*Bull. Soc. Chim.*, 1905 [ii], **33**, 1032, 1040, 1906 [iii], **35**, 14) have put forward the following equations: —



They further state that it has been shown that the composition of the gelatin does not alter after the hardening and therefore the oxygen is not absorbed, but probably liberated. It is difficult, however, to see why gelatin should behave differently from other organic compounds in this respect.

The equations given above cannot be proved experimentally. In all the laborious experiments carried out by Lumière and Seyewetz and others it has never been shown that the compound  $\text{CrO}_2$  is formed, but only that under the action of light the dichromate is affected in such a way that all the chromic acid cannot be extracted from the gelatin by treatment with water or with ammonia solution. From analogy with other reactions where organic substances are involved, this would indicate that the dichromate is reduced more or less completely to a stage of oxidation lower than that where the chromium is sexavalent, possibly to tervalent chromium; it may then be assumed, and it is only an assumption, that the tervalent chromium salt forms a chromium chromate with the dichromate or chromate left unaltered. The hardening of the gelatin may be due to oxidation, and possibly, in part, to the liberation of chromium hydroxide by hydrolysis of the chromium chromate, if this latter compound is assumed to behave like other chromic salts.

Independent of any theory of the process, Lumière and Seyewetz obtained the following results: —

- (1) The longer the exposure to light, the less soluble chromate can be extracted
- (2) At least a 0·1 per cent. solution of potassium dichromate is necessary to render gelatin insoluble in boiling water.

The quantities of chromium, calculated as  $\text{Cr}_2\text{O}_3$ , fixed by 100 grams of gelatin after exposure to light for the minimum time necessary to make the gelatin insoluble were:—

|                                |                                  |
|--------------------------------|----------------------------------|
| 0·1 per cent. dichromate ..... | 0·39 gm. $\text{Cr}_2\text{O}_3$ |
| 0·5      "      "      .....   | 0·87      "      "               |
| 1·0      "      "      .....   | 1·08      "      "               |

- (3) The quantity of chromium which is retained by gelatin made insoluble with potassium dichromate and exposure to light is about three times as great as when a chromic salt is used.
- (4) The action of light on gelatin impregnated with ammonium dichromate gives rise, in a given time, to a larger quantity of insoluble chromium compound than when potassium dichromate is used. After 10 hours there is more insoluble chromium compound formed with the ammonium salt than in 7 weeks with the potassium salt.

With chromic acid and the dichromates or chromates of ammonium, potassium, sodium, lithium, copper, zinc, barium, lead, aluminium or iron, using solutions containing an amount of chromium corresponding with that in a 3 per cent. solution of potassium dichromate, the amounts of chromium fixed in the gelatin are approximately the same. Also, a number of the gelatins made insoluble enclose oxides of the other metals, and it is noticeable that in the case of the iron salt the gelatin retains very little  $\text{Cr}_2\text{O}_3$  and much  $\text{Fe}_2\text{O}_3$  after extraction with ammonia solution. In the case of other salts of the heavy metals, some of the oxide is retained by the gelatin.

- (5) Dichromated gelatin which, after keeping 1·4½ months in the dark, has become insoluble, contains smaller quantities of insoluble chromium compounds than does the same gelatin when exposed to the light, and is also less resistant to boiling water. If it is made insoluble by keeping at 120° in the dark for 1·6 days, it is quite insoluble in boiling water, but is broken up by it into small fragments.

Namias (*Phot. Korr.*, 1902, **39**, 446) states that gelatin hardened with dichromate can be washed colourless in 24 hours, whereas that hardened with chromium sulphate remains green; the colourless gelatin in the former case still contains detectable quantities of chromium. This does not agree with Lumière and Seyewetz's statements given above. Abegg and von Schroeder (*Kolloid Zeitschr.*, 1907, **2**, 85) state that successive treatment in baths of potassium dichromate and sodium thiosulphate, and also in solutions of tannin or picric acid, tan gelatin with lowering of the melting point.

According to Gamble (*J. Soc. Chem. Ind.*, 1910, **29**, 65)  $\alpha$ -gelatin yields a maximum amount of insoluble matter when exposed to light in conjunction with an alkali dichromate. The yield of insoluble matter with  $\beta$ -gelatin is less, whilst the gelatin peptone does not give an insoluble product.

Hans Meyer (*Zeitschr. physikal. Chemie*, 1909, **66**, 33) has measured the electrical conductivity of dichromated gelatin in the dark and in the light, and found that the conductivity decreases considerably on exposure to light, but his experiments do not lead to any conclusions as to the mechanism of the process.

In the account of Lüppo-Cramer's work given in the earlier part of this section the effect of oxalates and citrates was mentioned. Namias (*Eder's Jahrbuch*, 1904, 142; 1906, 125) found that the addition of oxalates and citrates to dichromated gelatin films rendered them stable for a long time, whereas they would otherwise harden spontaneously after keeping for a short time in the dark. This has been confirmed by other workers (Eder, Hanneke, Harris, König).

Wilkinson (*Trans. Jour.*, 1911, **51**, 387) has investigated the various reticulations of gelatin obtained by the use of sodium, potassium and ammonium chromates and dichromates, and of mixtures of these salts. Compare also Sheppard and Elliott (*J. Ind. Eng. Chem.*, 1918, **10**, 72).

#### *Hardening Action of Organic Compounds on Gelatin.*

It has long been known that during the development of a photographic plate hardening of the film may occur. Haddon and Grundy (*Brit. Jour. Phot.*, 1896, **43**, 356) found that pyrogallol solution had no tanning effect on gelatin, but that when the solution became brown through oxidation it exerted strong tanning action and the gelatin became insoluble in hot water. Observations on other photographic developers were also made. Lüppo-Cramer, using the developer adurol, found that when the quantity of sulphite in the solution was very small the developed plate showed considerable relief owing to the tanning of the gelatin, which was especially marked in the high lights. The tanning was ascribed to the oxidation products of the adurol.

Apparently in ignorance of the above-mentioned results, Lumière and Seyewetz (*Bull. Soc. Chim.*, 1906, [iii], **35**, 377, 600, 1907, [iv], **1**, 428; 1908, [iv], **3**, 743) carried out a number of experiments on similar lines. In their first paper the developers tested were:—pyrogallol, hydroquinone, pyrocatechol, *p*-aminophenol, diaminophenol hydrochloride, diaminoresorcinol hydrochloride, metol-quinol, adurol, *p*-phenylenediamine, eikonogen, metol and glycine. They were used (1) in 1 per cent. solutions, (2) in 1 per cent. solution + 3 per cent. sodium carbonate, (3) in 1 per cent. solution + 3 per cent. sodium carbonate + 3 per cent. sodium sulphite. Strips of gelatin were immersed in these solutions (*a*) in sealed flasks, (*b*) exposed to the air.

The results show that gelatin only becomes insoluble (after 1-6 days) when the developers are in the presence of alkali and in contact with the air, and that probably the active agents in rendering the gelatin insoluble are the oxidation products formed from the developers. Where the solutions are not exposed to the air the gelatin will readily dissolve even after a month; also, where the oxidation product is insoluble in dilute alkalies, as in the case of *p*-aminophenol, the action is slight. In a negative the action is usually most marked in the neighbourhood of the reduced silver, and this is ascribed to the more rapid oxidation of the developer in that region by the bromine liberated.

Under ordinary circumstances, that is "during development of a negative," hardening only takes place with pyrogallol as developer. This is probably because the other developers, in the presence of sulphite, only oxidise very slowly in the air.

In the presence of developer *plus* sodium carbonate, gelatin did not harden after one month, when *p*-phenylenediamine and glycine were used. When sodium sulphite was present in addition to the sodium carbonate, the gelatin became insoluble only with the developers:—pyrogallol, diaminophenol and diaminoresorcinol.

A number of phenols which do not act as developers were examined with respect to their hardening action on gelatin; these included:—phenol, *p*-cresol,  $\alpha$ - and  $\beta$ -naphthol, resorcinol, gallic acid, tannin (gallotannic acid), dihydroxynaphthalene, phloroglucinol, salicylic acid, *p*-nitrophenol, sodium  $\alpha$ -naphthol-4-sulphonate, and sodium  $\beta$ -naphthol-3-6-phenol sulphonate. In the presence of sodium carbonate and in solutions exposed to the air only the following made the gelatin insoluble:—gallic acid, tannin,  $\alpha$ -naphthol,  $\beta$ -naphthol, resorcinol, phloroglucinol and dihydroxy-naphthalene; gallic acid and tannin were effective also in the presence of sodium sulphite. The time necessary for hardening varied from 2-5 days, except for  $\alpha$ -naphthol (25 days) and resorcinol (45 days). Comparing the

time necessary for resorcinol with the times necessary for its two isomerides, hydroquinone and pyrocatechol, which were respectively 1 and 2 days, it will be seen that the fact that the two latter are developers plays an important part.

In proof of the statement that it is the oxidation product of the developer which hardens the gelatin, it was shown that quinone (benzoquinone), whether added to the solution or to the solid, will render gelatin insoluble in cold water. The action proceeds all the more slowly the more dilute the solution of quinone, but once the gelatin is set it no longer melts on warming, nor will it dissolve in water after prolonged boiling. It is impossible to regenerate the gelatin and the quinone from the product, which is not dissociated into its constituents by acids, alkali carbonates or hydroxides or by ammonia.

Trunkel (*Biochem. Zeitschr.*, 1910, **26**, 458) has investigated the precipitation of gelatin by tannin. In aqueous solution tannin and gelatin will give complete mutual precipitation under definite conditions of concentration. With excess of tannin above this amount there will be tannin in the filtrate. With excess of gelatin, both tannin and gelatin will be found in the filtrate. In connection with such precipitation reactions it is stated that the same quantity of gelatin (1 gram) requires more tannin for precipitation (0.7 gram) when fresh than when the solution has stood for 24 hours (0.4 gram), so that an ordinary precipitation reaction may be used to show the effects of previous history on the gelatin.

The reactions with tannin have been more thoroughly investigated by von Schroeder (*Kolloid Beihefte*, 1909, **1**, 1; compare also Ricevuto, *Kolloid Zeitschr.*, 1908, **3**, 114; Levites, *Kolloid Zeitschr.*, 1911, **8**, 4). Since gelatin is an amphoteric colloid, and tannin is a negative colloid, it follows that gelatin will only be precipitated by the tannin when it (the gelatin) is positive. This is found to be the case; carefully dialysed gelatin is not precipitated, nor is gelatin which has been made neutral or slightly alkaline by the addition of sodium carbonate. When the gelatin is made acid, however (that is on the acid side of the isoelectric point), precipitation takes place. Ricevuto found that precipitation of the gelatin by tannin was again prevented as soon as the added acid reached a certain concentration, but von Schroeder found that in the case of hydrochloric acid (also nitric and sulphuric acids), precipitation is not prevented at any concentration between 0.001-N and 0.25-N in a solution containing 0.5 per cent. gelatin and 0.75 per cent. tannin. Experiments with acetic, mono- and di-chloroacetic, succinic, and citric acids, showed that their retarding effect on the precipitation is the greater the greater the strength of the acid (qualitative results only). Trichloroacetic acid could not be used, since it precipitates gelatin by itself.

Von Schroeder also found that after keeping the gelatin sol for 24 hours higher concentrations of acid were necessary to prevent precipitation by tannin than with the fresh sol, the influence of the previous history of the gelatin thus again playing a part.

It was proved by investigation of what is known as the "adsorption isotherm," that the reaction between gelatin and tannin is a typical adsorption process.

#### *Gelatin and Formaldehyde.*

The first quantitative investigation of the action of formaldehyde on gelatin seems to have been made by Lumière and Seyewetz (*Bull. Soc. Chim.*, 1906, [iii], **35**, 872). When plates of gelatin are soaked in a 10 per cent. solution of formaldehyde the insoluble product formed contains from 4.48 gms. of the aldehyde per 100 grams dry gelatin as a maximum (after 12 hours' soaking). The rapidity with which the formaldehyde is absorbed under these conditions increases with the concentration of the formaldehyde solution (up to 10 per cent. H.CO<sub>2</sub>H), and according to Abegg and von Schroeder (*Kolloid Zeitschr.*, 1907, **2**, 85) the velocity

follows that of a reaction of the first order, except at very small concentrations, where the degree of tanning is less than would be calculated. Levites (*Kolloid Zeitschr.*, 1908, **2**, 237) also found that in the presence of much water the reaction between gelatin and formaldehyde takes place slowly (compare also Reiner, *Kolloid Zeitschr.*, 1920, **27**, 195). The facts that the results are in accordance with a unimolecular reaction does not prove that the phenomenon actually measured is chemical in nature; it may be physical, as indeed seems probable from the fact that the velocity was not sensibly influenced by the increase of temperature from 17°-50° (Lumière and Seyewetz).

According to Brotman (*J. Soc. Leather Trades Chem.*, 1921, **5**, 363) the amount of formaldehyde fixed by gelatin is a function of the concentration of the gel, a weak gel fixing more formaldehyde than a more concentrated one under the same conditions.

Gaseous formaldehyde is absorbed more slowly by gelatin than is the solution, but the maximum amount absorbed is the same in both cases.

The reaction is a reversible one, since gelatin which has been rendered insoluble by formaldehyde may be restored to its original condition by repeated treatment with hot water (Lumière and Seyewetz) or by digesting at 100° with a little water (Reiner). Abegg and von Schroeder state that a non-melting gelatin, tanned with formaldehyde, had a melting point of 48° after washing for 48 hours with cold water, and a m.p. of 46° after 55 hours' washing, the m.p. of the untanned gelatin being 36°. Formaldehyde is also gradually liberated from the insoluble product when the latter is heated at 110° (Lumière and Seyemetz).

Maceration with cold dilute hydrochloric acid will remove formaldehyde from the hardened product (Lumière and Seyewetz), and the hardening process may be entirely stopped and reversed by the addition of small quantities of ammonia (Reiner). Consequently by using calculated quantities of ammonia, hardening may be stopped at any point.

Hardened gelatin loses its power of melting, but the mass still softens at elevated temperatures, the softening temperature is dependent on the amount of hardening and the concentration of the gelatin (Reiner). The hardened gelatin softens at temperatures higher than the melting point of the untreated material. In contradistinction to untreated gelatin, softening of the hardened product commences at the centre and spreads through the mass, and, on raising the temperature sufficiently, the gelatin becomes liquid. If the softening temperature lies above 90°, then complete liquefaction is no longer possible. The hardening appears to occur more completely on the surface than in the interior of the mass. On cooling a melted, hardened gelatin, the setting point lies at, or in most cases below, that of the untreated gelatin.

The above facts are explained by Reiner as follows (Lumière and Seyewetz gave a similar explanation):—Formaldehyde does not attack gelatin uniformly; one part is hardened whilst the other remains unattacked. The hardened part forms a more or less coherent structure, and on continued action becomes much more coherent and encloses the unhardened part of the solution. In this way the gel loses its characteristic melting point, but if the structure is disturbed by melting, then the mass solidifies at the point of solidification of the unhardened gel. Compare also, Moeller (*Kolloid Zeitschr.*, 1919, **25**, 67; 1921, **28**, 281, 1921, **29**, 45).

Abegg and von Schroeder (*loc. cit.*) state that the viscosity of a dilute solution of gelatin (1-2 per cent.) diminishes with time after the addition of formaldehyde, but no reference is made to such an effect by Bogue (*J. Chem. Ind. Eng.*, 1920, **23**, 5) in his experiments on viscosity.

According to Abegg and von Schroeder the tanning action of 1 per cent. formaldehyde is not affected by the presence of 20 (vol.) per cent. alcohol, whilst it is considerably accelerated by the addition of sodium carbonate.

Levites (*Kolloid Zeitschr.*, 1908, **2**, 237) tested a large number of salts (6 per cent. gelatin, 2 per cent. HCOH and normal solution of the salt) at 50°, 60° and 70°, and found that all the salts retarded the velocity of gelatinisation.<sup>t</sup> The retardation is all the greater the lower the temperature and the slower the reaction normally proceeds. At 100° the salts have practically no action. Salts of bivalent ions have a greater influence than those of univalent ones, e.g., calcium chloride greater than sodium chloride. Within certain limits the action of the salts of univalent ions seems to be independent of their concentration, whilst molecular solutions of salts with ions of the same valency have approximately the same retarding effect. Glycerol, glucose and mannite also retard.

Both hydrions and hydroxyl ions have an effect, the former accelerating and the latter retarding gelatinisation. The action of both ions increases with the concentration, that of the hydroxyl ions being much more marked than that of the hydrions, although both effects are very great.

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### JELLY-STRENGTH OF GELATIN

Until the last few years no attempts seem to have been made to connect the so-called "jelly strength" of gelatin with other properties. In the early days, measurements of the elastic properties of gels were made purely from the physical point of view, the first gelatin which came to hand being used, and no account taken, or description given, of its chemical and other properties; in very many cases the kind of gelatin used is not stated.

The early investigations had reference chiefly to the measurements of the elasticity coefficient and of Poisson's ratio,  $\mu$ . Poisson's ratio is the ratio of unit increase in length to unit decrease in diameter ( $\Delta l/l \div \Delta d/d$ , where  $l$  = length and  $d$  = diameter) when a rod of gel is stretched. If the rod suffers no alteration in volume when it is thus stretched,  $\mu$  should have a value of 0.5, and this was found to be the case by Wiedemann (*Verh. phys. Gesellsch. Berlin*, 1884, p. 45), Maurer (*Wied. Ann.*, 1886, **28**, 628) and Fraas (*Wied. Ann.*, 1894, **53**, 1074).

The compressibility of a gel depends on its elasticity, and de Metz (*Wied. Ann.*, 1890, **41**, 663) found that the compressibility decreased with the age of the gel, that is, the elasticity increased, an observation which was first made by Sir D. Brewster in 1815 in connection with some experiments on the double refraction of plates of gelatin (*Phil. Trans.*, 1815, p. 60). The coefficient of compressibility with increasing pressure was found to be greater than that for decreasing pressure. Von Bjerken (*Wied. Ann.*, 1891, **43**, 817) showed that when a strip or rod of gelatin gel is stretched beyond a certain limit it contracts on warming; under the same conditions it should become warmer on being further stretched, and this was found to be the case. It is of interest to note that Bjerken states in his paper that "the more accurate composition of the gels has very little interest, but it may be stated that they all consisted mostly of liquid (at the most 20 per cent. gelatin)."

Reference may also be made to a paper by Rankine (*Phil. Mag.*, 1906, **11**, 447) on the decay of torsional stress in gelatin gels, and to various papers which deal more particularly with the existence of structure in very dilute solutions of gelatin (Schwedoff, *J. de Physique*, 1889, **8**, 341, Colin, *Comptes Rendus*, 1893, **116**, 1251; Rohloff and Shinjo, *Physik. Zeitschr.*, 1908, **8**, 442).

In the course of the above investigations various observations were made which are of interest, one of which has already been mentioned in connection with the effect of age on the compressibility. Fraas and Rankine (*loc. cit.*) each point out that the previous history of the gel is important and must be taken into account before measurements are made. Fraas found that the elasticity coefficient of pure gelatin gel decreases rapidly with increase in concentration of the gelatin between 10 and 50 per cent. Sodium chloride (also potassium and calcium chlorides) decreases the elasticity and rigidity, so much so that rods of gel, 30 cm long and 15.7 mm. in diameter, containing 20 per cent. of sodium chloride, break under their own weight. Glycerol, so long as it is not present in sufficient quantity to affect the solubility of the gelatin, has no effect on the elasticity finally obtained. Sucrose and gum arabic behave similarly to glycerol. Leick (*Wied. Ann.*, 1904, **14**, 139), using a photographic gelatin, investigated the relation between elasticity and concentration somewhat more accurately and found that the elasticity coefficient (Young's modulus) is approximately proportional to the square of the concentration of the gelatin, except at high concentrations (30 per cent. or more), where it increases more slowly than the square of the concentration. He also confirmed Fraas's observations that sodium, potassium and calcium chlorides, as also lithium and magnesium chlorides and potassium nitrate, diminish the value of the elasticity coefficient, lithium, calcium and magnesium chlorides having a specially strong influence in this respect. Sodium sulphate has practically no effect. Contrary to Fraas, however, he finds that glycerol and sucrose increase Young's modulus considerably.

concentration is not confirmed. If the relation is of the form  $E = kC^n$ , then it should follow that  $\log E = \log k + n \log C$ , that is, a straight line relation between  $\log E$  and  $\log C$ . This is found to be the case for some gelatins, but with others the curve is definitely concave to the  $\log C$  axis. When there is a straight line curve the value of  $n$  may be less than or equal to 2.

It was further found that the changes of elasticity with acid or alkali are not simple functions of the pH of the gel.

With increasing alcohol content up to about 25 per cent. the modulus of rigidity and the jelly strength increased slowly at first. Up to about 40 per cent. the gels broke normally, but above this concentration the elastic limit was reached and alcohol and water were squeezed out of the gel.

Preliminary experiments only were made of the effect of glycerol.

It has been pointed out that the jelly strength of a gelatin is not dependent on the concentration according to a simple relation, since the curves showing the relation between the jelly strength, as measured by the breaking load or by the modulus of rigidity, and the concentration for different gelatins cut each other. The same thing holds for the curves showing the relation between the setting point and the concentration (Sheppard and Sweet, *J. Ind. Eng. Chem.*, 1921, **13**, 423). Moreover, the order for different gelatins, if graded according to the setting point, would not necessarily be the same as if their jelly strengths were used.

The most direct method of adjusting these difficulties would be to compare the integral values of the jelly strength—concentration curves or setting point—concentration curves respectively, over the complete range of 0-100 per cent. concentration. This, however, is not possible, but Sheppard and Sweet suggest that the comparison be made for a lower range, up to 25-50 per cent. concentration. The areas enclosed by the corresponding curves and the axes of coordinates may be measured either by a planimeter or by weighing. Results such as the following are then obtained. The figures are given in order of decreasing magnitude.

| Jelly strength values. |     |            |  | Setting point values. |     |            |  |
|------------------------|-----|------------|--|-----------------------|-----|------------|--|
| No.                    |     | Rel. area. |  | No.                   |     | Rel. area. |  |
| 3                      | ... | 697        |  | 1                     | ... | 1345       |  |
| 2                      | ... | 615        |  | 3                     | ... | 1320       |  |
| 1                      | ... | 321        |  | 2                     | ... | 1297       |  |
| 4                      | ... | 146        |  | 4                     | ... | 922        |  |
| 5                      | ... | 96         |  | 5                     | ... | 898        |  |
| 6                      | ... | 56         |  | 6                     | ... | 869        |  |
| 17                     | ... | 878        |  | 14                    | ... | 1550       |  |
| 14                     | ... | 610        |  | 17                    | ... | 1515       |  |
| 13                     | ... | 537        |  | 13                    | ... | 1478       |  |
| 16                     | ... | 356        |  | 15                    | ... | 1461       |  |
| 18                     | ... | 306        |  | 18                    | ... | 1437       |  |
| 15                     | ... | 294        |  | 16                    | ... | 1422       |  |

These tables show that there is much less difference between individual setting point values (the same holds for melting point values) within the range of concentration tested, than between the jelly strength values for the same sets of gelatin. Also, the order in any set is not the same for both values, that is, the grading of gelatins would give different results according to the method used. Each type of grading therefore seems to be desirable for adequate characterisation.

## ADDENDUM

Although not giving a direct measure of jelly strength in terms of coefficients of elasticity, experiments carried out by G. R. Smith (*J. Amer. Chem. Soc.*, 1919, **41**, 135; *J. Ind. Eng. Chem.*, 1920, **12**, 878) have indicated that there is a connection between the mutarotation of gelatin and its power of gelation. By mutarotation in the case of gelatin is meant the change in rotation which a solution or gel undergoes when the temperature is altered.

Very little study of the rotatory power of gelatin appears to have been made prior to the work of Smith. Trunkel (*Biochem. Zeitschr.*, 1910, **26**, 493), referring to the work of de Bary (*Hoppe-Seyler's Med.-Chem. Unters.*, 1866, **1**, 71), Kruger (*Magl's Jahressb. u. Fortschr. d. Tierchemie*, 1889, p. 29) and Frazee (*Archiv ges. Physiol.*, 1887, **68**, 141), states that the extent of our previous knowledge on the subject may be summarised as follows:—the specific rotatory power of gelatin changes with the temperature, and continued heating at 100° gives a product,  $\beta$ -gelatin, the specific rotatory power of which is lower than that of ordinary gelatin.

Trunkel used very dilute solutions of gelatin, which were prepared by heating for 6-8 hours at 80°, so that undoubtedly considerable hydrolysis had taken place, and therefore the conclusions he drew from his results are of very little importance, the same holds for the calculations which Wo. Ostwald (*Kolloid Zeitschr.*, 1915, **17**, 113) made from Trunkel's results with respect to the kinetics of the mutarotation of gelatin (cf. also Razumkin and Braudo, *J. Russ. Phys. Chem. Soc.*, 1915, **47**, 1333, 1916, **48**, 269).

In the experiments made by Smith the solutions were prepared from the powdered air-dried samples by soaking for a few minutes with water in a graduated flask, heating on the steam bath for 10 minutes, cooling and making up to volume at 35°. Prolonged heating and temperatures higher than 60° were avoided. Most of the readings were made when the solution was in the condition of a gel in the polarimeter tube.

With solutions in which the concentrations were 1, 2, 3, 5, and 7 grams of gelatin per 100 cc., extended measurements were made, sufficient time being allowed for the readings to become constant at the given temperature; the temperature varied between 10° and 45°. Above 35° constant rotation is quickly obtained if the solution, after preliminary heating to 36°-40°, is brought to the desired temperature; at lower temperatures the solutions must be kept for 10 to 15 hours at the desired temperature for the rotation to become constant. Constant rotation is the more quickly obtained in the more concentrated solutions.

At a temperature above 35° the specific rotatory power is practically constant, even with widely varying concentrations; another and quite different specific rotatory power is shown at 15°, which is again constant for the varying concentrations; similarly for 10°. The rotation increases with fall in temperature.

Since the "specific rotatory power" does not vary appreciably above 33°-35°, Smith concludes that there are two chemical forms of gelatin, one of which is stable above 33°-35° (sol form A), whilst the other form (gel form B) exists at lower temperatures. The transformation into the B form is practically complete at 15°. The condition at any intermediate temperature between 35° and 15° is one of equilibrium, which may be represented by the equation



The change in rotation being reversible with the temperature. The velocity with which the change in rotation takes place at, say 20°, when a solution at 35° is cooled down rapidly and maintained at 20°, showed that the time required for half the total change in rotation to take place was inversely as the concentration of the solution, indicating that the reaction is bimolecular. Velocity constants at 20°, calculated on this

assumption, gave good results, so that the reaction which takes place may be considered to be the combination of 2 mols. of A to form one mol. of B.

By measuring the equilibrium rotations at 24°, 25°, 26°, and 27° of 1, 2, 3 and 5 per cent. solutions, and knowing the constant rotations at 35° and 17° respectively, it was possible to determine the equilibrium constant of the above reaction.

Other evidence is given by Smith for the existence of two forms of gelatin. At temperatures below 30° gelatin solutions are either precipitated or made opalescent by the addition of alcohol to the extent of 15 per cent. When opalescence is produced a drop of a solution of alum or a feeble electric current will produce coagulation. Above 35° precipitation requires very much larger proportions of alcohol, about 40-45 per cent., unless a comparatively large amount of some electrolyte is added (*cf.*, Ilford Ltd., Renwick and Storr, *Eng. Pat.*, 16703/1915; 102168/1915).

For different concentrations of gelatin there are minimum temperatures above and below which the sol or gel condition respectively is stable for any length of time. Smith considers these temperatures to be the true melting points, and determines them as follows:—The sol is cooled 2°-3° below the expected temperature, maintaining the temperature at this point until the gel is produced, and then transferring to a constant temperature bath at the expected temperature. At this temperature the gel should show the selected condition of viscosity continuously for several (3-4) hours. For the measurements polariscope tubes were used, and the viscosity selected to mark the transition from sol to gel was such that a bubble of air, about 4.5 mm. in diameter, admitted to the tube, moved vertically with a scarcely perceptible motion of about 1 cm. in 4 secs. The minimum concentration of gelatin required to give a solution of such viscosity at a given temperature is said to be the concentration of a "standard gel."

The curve showing the relation between the concentration and the maximum gelation temperature was found to be of the usual form, approaching asymptotically to a temperature of 35°.

In the paper in the *J. Ind. Eng. Chem. (loc. cit.)*, Smith gives the results of a large number of measurements of the rotatory powers of different bone and hide gelatins at 35°, 15° and 10°, and discusses the relations which exist between the mutarotation and the jelly strength, the latter being defined in terms of the standard gel. It is found that as the minimum amounts of gelatin necessary to give a standard gel decrease, the mutarotation, the rotation per gram at 15° and 10°, and the ratio of the rotations at 15° and 35° all increase.

## APPENDIX II

### THE BEARING OF RESULTS OBTAINED IN RECENT INVESTIGATIONS OF SOAP SYSTEMS UPON THE STRUCTURE OF GELATIN GELS

At first sight there would not appear to be any marked similarity between soaps on the one hand and gelatin on the other; they have, however, a number of similar properties. Thus, like soaps, gelatin forms, under suitable conditions, colloidal solutions (clear fluid sols) and transparent elastic gels, and it is probably also capable of existence in a form analogous to curd. The similarities in physical behaviour are in fact, so close that any hypothesis put forward to account for the behaviour and structure of gelatin must also be capable of application to soaps. It is possible, indeed, that knowledge of the structure of gelatin gels will be gained by the study of soaps since the latter are of definite, known composition and are obtainable in the pure state with relatively little difficulty. On the other hand the chemical structure of gelatin has not yet been completely elucidated and, as the foregoing report shows, it is most difficult to obtain the material itself in a state of purity. For experimental purposes then, soaps are more tractable materials than gelatin. Further, the study of soaps has now reached the stage when it is possible to cause the solutions to reproduce their characteristic properties at will by carefully controlling all the factors concerned. A similar stage is also being approached (but can hardly be said to have been reached) in the study of gelatin as is suggested by the following statement of C. R. Smith (*J. Amer. Chem. Soc.*, 1921, **43**, 1365). .-

"The results of this paper are quantitatively reproducible. As the study of the properties of gelatin has advanced, little excuse is seen for applying the term 'hysteresis' to the failure to reproduce conditions and results."

In the following paragraphs such of the more important results obtained in the recent studies of soap systems as appear to have a bearing are briefly recited and their utility in a possible interpretation of the structure of gelatin gels is explained.

Soap has been shown by Miss Laing to be capable of existence in three typical states, i.e., as clear fluid sol (solution), transparent elastic gel (jelly) and white opaque curd. Under proper conditions and by suitable manipulation, it is possible to cause the soap in a particular solution (e.g., a moderately strong solution of the soap sodium oleate) to assume any one of the above three forms, further, it is possible to keep, side-by-side, three portions of the same soap solution at the same temperature, in which the soap is to be found in the sol, gel and curd states respectively. The sol and gel are clear and transparent, showing no traces of solid structure under the ultra-microscope, while the curd is revealed as a mass of fine white fibres enmeshing a very weak soap solution or almost pure water. The curd, the white fibrous structure of which is visible in the ultra-microscope and occasionally in the microscope, is the relatively insoluble form in which the soap has separated from solution. .

The curd state of soap is very markedly different from the sol and gel states, but the two latter states are strikingly similar. The soap solution, in fact, is turned into a transparent jelly without alteration of any of its properties except those of rigidity and elasticity. With this exception, the sol and gel states of soap are identical. This remarkable identity is found not only in those properties of the sol and gel which depend upon their osmotic pressure but also in their electrical conductivity. It should here be noted that the identity of several properties in the sol and gel states is observed in gelatin as well as in soap solutions. There has been much confusion in the past in regard to soap owing to failure to

discriminate between the phenomena of gelatinisation and curd formation; the two are entirely different and can be clearly distinguished by experimental means.

According to the ionic dissociation hypothesis, the molecules of salts in aqueous solution are supposed to be split up in part, into ions or radicles carrying electrical charges, and it is the migration of the electrically charged ions which enables such solutions to conduct electricity. Similarly soaps, which are essentially the alkali salts of fatty acids, are partly dissociated in aqueous solution. Thus a dilute solution of the soap sodium stearate contains not only undissociated soap, but also positively charged sodium ions and negatively charged stearate ions. In more concentrated solution the stearate ions agglomerate to form particles or micelles which are colloidal in character and yet retain the negative electrical charges of the unit ions of which they are composed. Similarly the undisassociated soap agglomerates to form particles which are colloidal but electrically neutral. When a soap solution becomes gelatinised (transformed into a gel) it is clear that the gel structure must be made up either of the colloidal neutral soap or of the colloidal ionic micelles. The evidence at present available would seem to indicate that it is the neutral colloidal particles or micelles of undisassociated soap which are linked up to form the gel framework. In any case the same constituents and the same amount of the same actual particles exist in sol and gel.

Returning to the general problem of gel formation, it may be said that five explanations have been put forward to account for the properties of gels. These explanations may be described as:—

- (1) a molecular network throughout the gel;
- (2) a closed honeycomb of solid enclosing liquid;
- (3) a porous, but continuous, solid cellular framework;
- (4) a more or less rigid emulsion composed of two liquids;
- (5) the micellar theory; that is a structure probably fibrous made up of particles or micelles. The soap solution is supposed to be filled with innumerable fibres of this sort, whilst still containing the ions and ionic micelles as outlined above.

The identity of many and significant properties of sol and gel has already been emphasised. The conclusion to be drawn from this identity is that it is impossible for there to be any but very slight differences in the physical states of sol and gel. In each state a sensitive physical and chemical equilibrium exists and these equilibria must be identical in sol and gel since any slight change in them would lead to marked differences in such properties as electrical conductivity and osmotic pressure effects.

Various investigators, selecting one or other of the five above hypotheses concerning the phenomenon of gelatinisation, have agreed that the latter is, in effect, a process of crystallisation. The evidence gained from the study of soap systems is, however, incompatible with this suggestion if we consider crystallisation to be the removal of substance from colloidal solution. The process of gelatinisation is such a subtle one that the colloidal particles are not sufficiently removed from the solution as to disturb the equilibrium existing in the latter. This is not in accordance with hypotheses (2) and (3).

The experimental results which have been outlined have an important bearing upon the five explanations put forward to account for the properties of gels. The first explanation suggests that gels contain, as part of their structure, a network which is in effect one linked-up system of molecules; the rest of the solution consists of simple molecules and ions. In the investigation of soaps, however, it is quite clear that the colloidal properties cannot be ascribed to the presence of hypothetical unknown molecules of great complexity and very high molecular weight, since the chemical formulae and molecular weight are known. While the soap is in true solution it behaves like an ordinary, non-colloidal salt.

The electrical conductivity of a soap sol is quantitatively the same as that of the gel obtained from it. Now if a sol is changing to a gel became enclosed within solid cells or a cellular framework or became emulsified with what is essentially a different liquid, the electrical conductivity could not remain unchanged in the face of such fundamental changes in the conditions. Thus the hypotheses (2), (3) and (4) above would seem to be like (1), untenable.

The suggested explanation (5) assumes that the particles become stuck together, or arranged in loose aggregates which may be granules or, more probably, threads similar in character to the thread-like strings of particles of cadmium or iron oxides formed when an arc discharge passes between cadmium or iron poles in air. This explanation, which is called the micellar theory, is in harmony with many characteristic phenomena exhibited by gels such as syneresis (exudation), double refraction, swelling, peptisation, the definite form and elasticity of gels, coagulation, dehydration and vapour pressure, pleochromism (the exhibition of different colours when viewed in different directions), and certain optical and ultra-microscopic phenomena, etc. In fact, all the characteristic properties of gels can be explained in the light of the micellar theory.

Finally this theory explains the puzzling fact that the apparent viscosity of a sol frequently depends upon its previous treatment and history. In many sols the process by which colloidal particles become linked up (with the ultimate result of gel formation) is in progress; the observed viscosity of the sol will depend upon the extent to which this process has progressed.

For these reasons the micellar conception of gel formation and structure must be regarded as, at least, a valuable working hypothesis.

[References: M. E. Laing and McBain (Trans. Chem. Soc. London, 1920, 117, 1506); Darke, McBain and Salmon (Proc. Roy. Soc., 1921, 98, A, 395); Third Report on Colloid Chemistry of the British Association, 1920, 2-31.]

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## APPENDIX I

## DESCRIPTIVE BIBLIOGRAPHY OF GELATIN

Towards the end of 1920 the Council of the British Photographic Research Association requested me to draw up a report summarising the work which had hitherto been published on the physical properties of gelatin, the properties to be taken into account being viscosity, swelling, setting point and melting point, coagulation and precipitation, hardening, and jelly strength. Since it was desirable that the report should be ready as quickly as possible it was decided that it was not then feasible to search photographic Journals, which are not well indexed, for stray observations on gelatin which may be scattered through the various papers; similarly for Journals dealing with the leather industry. The report, therefore, deals exclusively with papers, of which the existence could readily be found by reference to the ordinary indexes of scientific literature. Theoretical questions are dealt with in an elementary manner, complications which would occur in a fuller treatment of the subject being avoided.

A sufficient number of copies of the report for distribution to the members of the Association specially interested in the question of gelatin were prepared and sent out in July, 1921. It was felt however, by the Council of the British Photographic Research Association and by the Department of Scientific and Industrial Research that since there exists no other such summary at the present time it was desirable to give it a wider circulation. It has consequently been revised and is now issued under the aegis of the Department.

The endeavour has been made to take into account all the important papers dealing with the above-mentioned properties of gelatin, but, in the short time which has been at my disposal, time which has been largely occupied with other duties, and in view of the fact that articles on gelatin are widely scattered in the scientific literature of all countries, it is very improbable that no papers have been missed. I should be glad to receive details of any such omissions from anyone interested in the subject.

T. SLATER PRICE,  
Director of Research,  
British Photographic Research Association.

February, 1922.

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## INTRODUCTION

In the early days of the investigation of gelatin with respect to the properties of swelling, viscosity, etc., it was usual to consider the gelatin purely as a colloidal substance and take little or no account of its chemical properties. This held not only for gelatin itself, but also for the influence of acids, bases and salts on the physical properties mentioned, and as a result there has accumulated a vast quantity of disconnected data. In recent years, however, it has been recognised that gelatin has definite chemical properties as an acid or as a base, and that these properties affect its behaviour in the various reactions to which it is submitted. Recognition of this fact is of comparatively recent date, but already order is being brought out of chaos and explanations are being found for many phenomena which were previously inexplicable.

It is true that during the last twenty years a number of authors, e.g., Bugarsky and Liebermann, Hardy, Pauli, Robertson, Sørensen, and others (compare Loeb, "The Physics and Chemistry of Colloids," Discussion held by the Faraday Society and the Physical Society of London, October 25th, 1920, Appendix I, pp. 153-161; also *Science*, 1920, **52**, 449-456) have advocated a chemical conception of the reactions of proteins, including gelatin, but since their results could be explained just as well on the basis of ordinary colloid chemistry, they were not generally accepted. Two chief reasons may be given for this non-acceptance:—

- (1) The experiments do not show that ions combine with proteins in the typical ratio in which the same ions combine with crystalloids.
- (2) The existence of the so-called Hofmeister series.

The Hofmeister series will be referred to in detail in later sections, but it may be stated here that they arose out of the investigations of Hofmeister and others on the effect of salts, etc., on the precipitation, viscosity, swelling, and other properties of proteins. It was found that the anions could be arranged in definite series according to their relative actions, the order being independent of the nature of the cation. Similar series were found for the cations, though these series seemed to be less definite. This may be illustrated by the following series, due to Pauli, which represents the relative effects of different acids on the viscosity of blood albumin:—

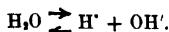
Hydrochloric > monochloroacetic > oxalic > dichloroacetic > citric >  
acetic > sulphuric > trichloroacetic acid,  
the hydrochloric acid increasing the viscosity most.

The order in which these acids are arranged bears no relation to their relative strengths, as judged by their dissociation constants. Other series could be quoted which give similar anomalous results, that is, anomalous in the sense that there seems to be no connection between the ordinary physical properties of the members of the series and the effect they have on the proteins. Because of these anomalies chemists were for a long time content to explain these series on the assumption of a selective adsorption of the various ions by colloids.

In the last few years, the conception of gelatin as an amphoteric substance, and the recognition of the fact that the behaviour of gelatin (and other proteins) must be compared at the same hydron concentration, has thrown new light on the subject and given an explanation of many phenomena which were formerly very puzzling.

It may be as well briefly to consider what is meant by the statement that gelatin is an amphoteric substance. Such a substance is one which is capable of functioning both as an acid and as a base; the most common and simplest example is water. The purest water that has been obtained

is still capable of conducting electricity to a very slight extent, that is, ions must be present. The only way in which ions can be formed is by the dissociation of water itself, in accordance with the equation :—



The water therefore contains both hydrions and hydroxyl-ions, that is, it is an acid and a base at one and the same time. Now a substance cannot function in this manner and be strong, either as an acid or a base. It must be a very weak acid and a very weak base, and in the case of water this is borne out by the fact that the dissociation is so small that in 10 million litres of water at 25° there are only one gram of hydrions and 17 grams of hydroxyl-ions. The water is neutral because the two kinds of ions are present in equivalent quantities.

Water, therefore, may function either as an acid or a base, and may be called an *amphoteric* substance.

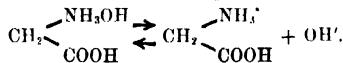
The most common amphoteric substances are organic compounds which contain both acid and basic groups in the molecules. Take, for example, aminoacetic acid,  $\text{NH}_2\text{CH}_2\text{COOH}$ . As an acid this will dissociate in accordance with the equation :—



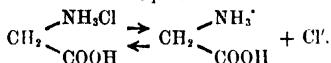
and with NaOH will form the sodium salt,  $\text{NH}_2\text{CH}_2\text{COONa}$ , which will dissociate in solution thus :—



The  $\text{NH}_2$ -group, however, confers alkaline properties on the compound, so that it will combine with hydrochloric acid to form a salt, the hydrochloride. It may be considered that when dissolved in water the  $-\text{NH}_2$  group combines with water to form the group  $-\text{NH}_3\text{OH}$ , just as ammonia,  $\text{NH}_3$ , when dissolved in water gives  $\text{NH}_4\text{OH}$ , and basic dissociation will then take place in accordance with the equation :—



With hydrochloric acid the hydrochloride will then be formed, which will dissociate in accordance with the equation :—



From its constitution it necessarily follows that water must be equally strong as an acid or as a base. In the case of aminoacetic acid (or as it is commonly called, glycine\*) the matter is different; it is about 100 times stronger as an acid than a base, the relative strengths being in the proportion of  $1.8 \times 10^{-10} : 2.7 \times 10^{-12}$  at 25°C. It follows therefore that when dissolved in water the solution will be acid, excess of hydrions,  $\text{H}^+$ , being present owing to the acid dissociation. If an electric current were passed through the solution the hydrions would wander to the cathode, whilst the negatively charged ion, the anion, would wander to the anode.<sup>†</sup>

It is a property of the dissociation equilibrium of water, that the products of the concentrations of the ions, that is  $[\text{H}^+] [\text{OH}^-]$ , where the square brackets indicate the concentrations, must be constant at a given temperature; the value of this constant is approximately  $10^{-14}$  at 25°C., the concentration of the individual ions (in pure water) being  $10^{-7}$  respectively. This must also hold when other substances are dissolved in

\* This must not be confused with the photographic developer *glycine*, which is a substituted amino-acetic acid.

<sup>†</sup> In this elementary treatment of the subject the complications due to the formation of internal salts, etc. are left out of consideration, so that the statements made are of a qualitative rather than of a quantitative nature. For a full discussion of the theory of amphoteric electrolytes, reference may be made to various papers by Sir James Walker (Proc. Roy. Soc., 1904, 73, 155; 1904, 74, 271).

the water. It follows that in the solution of glycine, where the concentration of the hydrions is much greater than  $10^{-7}$ , since the solution is slightly acid, the concentration of the hydroxyl ions must be less than  $10^{-7}$ , that is, the basic dissociation is very much suppressed. It will not be completely suppressed since there must always be sufficient OH' ions present to give, with the H' ions, an ionic concentration product of  $10^{-14}$ .

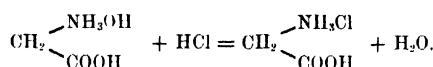
Suppose, now, gradually increasing quantities of hydrochloric acid are added to the solution; what will happen? This can best be understood by analogy. As is well known, when sodium acetate is added to a solution of acetic acid the acidity of the solution is diminished, a result which can be predicted theoretically and which depends on the fact that acetic acid is a weak acid, whilst sodium acetate, which contains an ion in common with acetic acid, namely the anion, is strongly dissociated. The diminished acidity of the solution is due to the throwing back of the dissociation of the acetic acid, whereby hydrions disappear. An exactly similar thing would happen to the acetic acid if the common ion, hydrion, were added to the solution, that is, if hydrochloric acid, which is strongly dissociated, were added. In contrast with the addition of sodium acetate to acetic acid, the necessity of adding hydrochloric acid to acetic acid in analytical practice would never arise.

Now return to the case of the addition of hydrochloric acid to a solution of glycine (aminoacetic acid). As has already been seen, in aqueous solution glycine dissociates mainly as an acid and only to a very slight extent as a base, that is, of the glycine ions which are present, the major

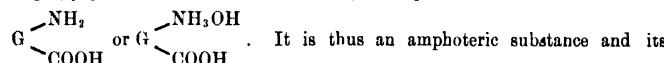
part consist of negative ions  $\text{CH}_2\begin{matrix} \text{NH}_3\text{OH} \\ | \\ \text{COO}' \end{matrix}$ , and only a very small part of positive ions,  $\text{CH}_2\begin{matrix} \text{NH}_3^+ \\ | \\ \text{COOH} \end{matrix}$ . On adding increasing quantities of hydrochloric

acid the dissociation of the glycine as an acid will be more and more suppressed and eventually a point will be reached where the acid dissociation has been so diminished that it has become equal to the basic dissociation, that is, there will be an equal number of positive and negative glycine ions in the solution. If an electric current were now passed through the solution, there would be no differential wandering of the glycine ions to the anode or cathode, that is, there would be no definite wandering with the current. When in this condition the glycine is said to be *isoelectric* and the solution is said to be at the *isoelectric point*.

If further quantities of hydrochloric acid are now added to the solution the acid dissociation of glycine is completely suppressed and the substance begins to function as a base, the hydrochloride being formed in accordance with the equation



Although the constitution of gelatin has not yet been worked out, it is known that it can function as an acid because of the presence of carboxyl, -COOH, groups, and as a base because of the presence of amino, -NH<sub>2</sub>, groups. In most cases it seems to act either as a monobasic acid or as a monoacid base, so that neglecting complications due to the presence of polypeptide linkages its formula may be represented thus:—

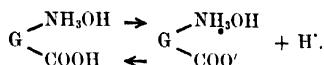



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\* It is not very probable that one and the same ion can possess positive and negative charges at the same time, that is, that an ion such as  $\text{CH}_2\begin{matrix} \text{NH}_3^+ \\ | \\ \text{COO}' \end{matrix}$  can exist.

behaviour will depend on whether the conditions are such that it functions as an acid or a base. (Brailsford Robertson has developed a theory that it is the polypeptide linkages,  $-\text{COH}:\text{N}-$ , in the molecule which confer acidic and basic properties on the proteins and has published a large book—"The Physical Chemistry of the Proteins"—on the subject; later work, however, seems to show that his theory is not valid.

As an amphoteric electrolyte, or, shortly as an ampholyte, gelatin is more acid than basic in character, so that in pure aqueous solution it will dissociate thus:—



The gelatin molecule (minus hydron), thus possesses a negative charge and should wander to the anode if a solution is electrolysed; this is found to be the case. If increasing quantities of acid, that is of hydron, are now added to the solution the gelatin should first change to the isoelectric condition, where it will not wander with the current, and afterwards should function as a base, so that it will be positively charged and wander towards the cathode. This is shown to be the case by the following experiments (Pauli, *Kolloidchemie der Eiweisskörper*, p. 33.) The experiments are probably due to Michaelis and Grineff (*Biochem. Zeitschr.*, 1912, **41**, 373). The concentrations of hydron ( $\text{C}_{\text{H}}$ ) given in the table are expressed in gram-ions per litre. (1 gm-ion of  $\text{H}^+$  weighs 1 gm., of  $\text{OH}^-$  weighs 17 grams, etc.)

| $\text{C}_{\text{H}}$    | Gelatin wanders to .. |    |    |     |     |               |
|--------------------------|-----------------------|----|----|-----|-----|---------------|
| $0.45 \times 10^{-5}$    | ...                   | .  | .  | ... | ... | anode.        |
| $0.9 \times 10^{-5}$     | ..                    | .. | .  | ..  | ..  | anode.        |
| $1.6-3.5 \times 10^{-5}$ | ..                    | .. | .. | ..  | ..  | no wandering. |
| $3.6 \times 10^{-5}$     | ..                    | .. | .. | ..  | ..  | cathode.      |
| $7.2 \times 10^{-5}$     | ...                   | .. | .. | ..  | ..  | cathode.      |

The mean of Michaelis and Grineff's results gives  $\text{C}_{\text{H}} = 2.5 \times 10^{-5}$  as the isoelectric point of gelatin.

Another way of stating this value of  $\text{C}_{\text{H}} = 2.5 \times 10^{-5}$ , which is now commonly used in the literature, is as follows: the logarithm of  $2.5 \times 10^{-5}$  is 5.39794, or -4.60206, i.e., 4.6. The isoelectric point is then said to have the value of  $\text{pH}$  or  $\text{pH} = 4.6$ . It is now commonly accepted that for gelatin the isoelectric point has  $\text{pH} = 4.6-4.7$ . Since the negative value of the pH is the logarithm of the concentration of the hydron it follows that the greater the numerical value of pH the less acid is the solution.

It can be shown (Michaelis, *Biochem. Zeitschr.*, 1912, **47**, 251; Sørensen *Ergebn. d. Physiol.*, 1912, **12**, 503) that at the isoelectric point the proportion of electrically neutral particles, that is, of undissociated molecules compared with the ions is a maximum. At such a point, therefore, gelatin or any other ampholyte, should possess very distinctive properties. Hardy (*Jour. of Physiol.*, 1899, **24**, 288) was the first to point out the peculiar behaviour of substances at the isoelectric point and showed that it was at that point that suspensoid colloids coagulate and precipitate.

### GELATIN AS AN AMPHOTERIC ELECTROLYTE

Before dealing specially with the properties of viscosity, swelling, etc., and the methods used in their measurement, it will be of advantage to detail some of the results obtained in investigations which have taken into account the amphoteric nature of gelatin. The investigators who have made noteworthy advances in this connection are Loeb (U.S.A.), Pauli (Austria) and Procter (England), and it will perhaps conduce to clearness if their results are dealt with separately, since, to a very great extent they have worked independently of each other. In the last few years Loeb has probably contributed most towards clarity of ideas on the subject of gelatin, and his work will therefore be dealt with first.

#### LOEB'S INVESTIGATIONS.

In a series of papers published in the *J. Biol. Chem.* (1917, **31**, 342; 1918, **33**, 531; 1918, **34**, 77, 394, 489; 1918, **35**, 497) Loeb called attention to the fact that the action of various salts on gelatin must be treated first as a chemical reaction, which reaction then determines the behaviour of the gelatin as regards its physical properties. In these investigations, however, no account was taken of the hydron concentration, that is, of the pH of the solutions used, so that it is not necessary to discuss them further. In all his later papers the pH of the solutions used has been determined and the values connected up with the various properties of gelatin. These later papers are to be found in the *J. Gen. Physiol.*, 1918-1919, **1**, 39, 237, 363, 483, 559; 1920, **3**, 85, 247; 1921, **3**, 391, and a summary of most of them is given in *Science*, 1920, **52**, 449.

Throughout his investigations Loeb makes use of a powdered, unbleached gelatin, in order to expose as large a surface as possible to the action of various solutions, so that equilibrium will be quickly obtained. The method of making isoelectric gelatin has been gradually improved, and the final methods used are as follows, the method chosen depending on the succeeding operations to be carried out.

(A) 25 grams of powdered gelatin having a pH of about 7.0, that is, of the same hydron concentration as CO<sub>2</sub>-free distilled water, are put into 1 litre of M/128 acetic acid for 30 minutes at 10°, after which time the acetic acid is renewed and left in contact with the gelatin again for 30 minutes at 10°. The acid is then decanted and replaced with distilled water at about 5°, after which the mixture is filtered in a Buchner funnel through muslin, using slight suction. The gelatin is then washed six times with 100 cc. of distilled water at 5° and made into a 5 per cent. solution (by warming with distilled water) which serves as a stock solution of isoelectric gelatin, the pH being about 4.7.

In using this solution for such measurements as viscosity, osmotic pressure, etc., some of it is heated to about 45° and made up to a 2 per cent. solution in quantity sufficient for a day's experiments, this 2 per cent. solution being kept during the day at 24°. To 50 cc. of this solution is added a sufficient quantity of the desired acid or alkali, the effect of which is to be investigated, and the volume is made up to 100 cc. by the addition of distilled water. The pH of each solution investigated is determined by Sörensen's indicator method, or by means of a gas chain.

(B) Dry powdered gelatin of pH = 7.0, and the grains no longer going through sieve 50, but going through sieves 40 and 30, is taken for the experiments. Quantities of one gram each of such gelatin are put for one hour in M/128 acetic acid at 10° to bring the gelatin to the isoelectric point. The mass is then put on a filter and washed five times with 25 cc. each of distilled water at 5°. In the weak acetic acid and during the washing the gelatin is stirred continuously. This isoelectric gelatin can then be used for swelling experiments in different solutions, or melted and used in 1 per cent. solution for viscosity and other measurements.

#### Isoelectric Gelatin.

A 1 per cent. solution of isoelectric gelatin is at first transparent. After some time, which is the shorter the lower the temperature, the solution becomes opaque and in the course of time (weeks or months) a precipitate may deposit. This does not happen in every case, possibly for

the reason that precipitation will only occur at a very definite pH, and with a slight deviation from this point on either side there will be only opacity at room temperature. Raising the temperature again gives a clear solution. The setting of the solution to a gel is a different process from this precipitation, since no cloudiness or opacity need be connected with this latter phenomenon.

At the Isoelectric Point gelatin practically does not swell, has a minimal viscosity, electrical conductivity, etc., and has practically no osmotic pressure.

From the consideration of gelatin as an amphoteric electrolyte, the isoelectric point of which is at pH = 4.7, it follows that in solutions of pH greater than 4.7 the gelatin will be present as an anion and will form salts with cations, giving the gelatinates; similarly, in solutions of pH less than 4.7 it will act as a cation and form salts with anions, as for example, with the chloride ion, giving gelatin hydrochloride. At the isoelectric point it has neither acid nor alkaline properties and therefore will not combine with either anions or cations. This has been proved as follows:—

Separate quantities of 1 gram each of powdered gelatin (going through sieve 60 but not through 80, and of pH about 7.0) were brought to different hydron concentrations by putting them for one hour at 15° into 100 cc. of nitric acid solutions varying in concentration from M/8, M/16, M/32, . . . down to M/8192. After filtering and draining, the separate portions were washed once or twice with 25 cc. of cold water at 5° or less, to remove remnants of the acid between the granules. The separate quantities of gelatin, which now possessed different pH's, were then put for one hour into beakers containing the same concentration of silver nitrate or sodium bromide, e.g., M/64, or M/16 or M/8, the temperature being 15°. They were then filtered and washed six to eight times, each with 25 cc. of ice-cold water, in order to remove the silver nitrate held in solution between the granules (the silver in combination with the gelatin is not removed by such washing, or at least only very slowly by altering the pH). Ice-cold water was used for washing since otherwise the granules would coalesce and the washing would be incomplete. Each quantity of gelatin was then melted at 40° and made into a 1 per cent. solution whose pH was measured, and which was then analysed for silver or bromine.

In the case of silver nitrate the results obtained are illustrated by the following figures:—

cc. 0.01N-Ag in combination with 0.25 gram of gelatin at different pH.  
pH ... 3.6 3.7 3.9 4.1 4.3 4.6 4.7 5.0 5.3 5.7 6.1 6.4  
cc. ... 0.5 0.3 0.3 0.2 0.2 0.2 0.2 0.55 1.25 3.2 4.0 4.85 4.9

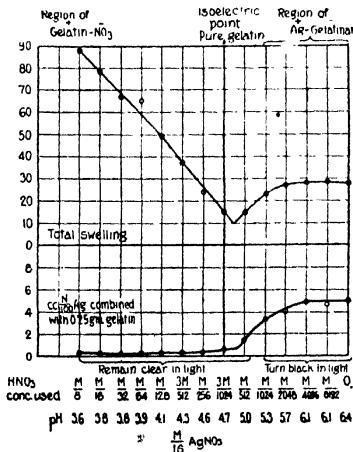


FIG. 1.

These results, which are plotted in Fig. 1, show that where the gelatin is present as anion, that is at a pH greater than 4·6-4·7, giving silver gelatinate, the silver is not removed by washing. If the washing had been more thorough there is no doubt that all the silver in concentrations with pH less than 4·7 could have been washed out of the gelatin. The retaining of the silver by the gelatin at pH greater than 4·7 is further well shown by the fact that if test tubes containing samples of the various gelatins are exposed to light, it will be found that those which are on the alkaline side of the isoelectric point blacken, whereas those on the acid side do not, but remain transparent even when exposed to light for months or years. (Fig. 2.)

Using sodium bromide it can be shown that bromine is retained on the acid side of the isoelectric point, owing to the formation of gelatin hydrobromide. (Of course, gelatin nitrate is formed on the acid side if silver nitrate is used, but this is not easy to show by analysis.)

cc. 0·01 N-Br. in combination with 0·25 gm. gelatin at different pH.

|         |     |     |     |      |     |     |      |     |      |     |     |     |
|---------|-----|-----|-----|------|-----|-----|------|-----|------|-----|-----|-----|
| pH 3·7  | 3·8 | 3·9 | 4·1 | 4·2  | 4·6 | 4·7 | 5·1  | 5·8 | 6·4  | 6·8 | 7·0 | 7·1 |
| cc. 7·5 | 7·4 | 7·1 | 5·0 | 2·95 | 1·4 | 0·1 | 0·15 | 0·2 | 0·15 | 0·2 | 0·1 | 0·1 |

Corresponding with these results it is found that if the properties of swelling, osmotic pressure, alcohol precipitation number, etc., are plotted as ordinates against the pH as abscissæ, the curves, as shown in Fig. 1, all show a sharp minimum at the isoelectric point.

Similar tests can be made for any other cation or anion the presence of which can be easily demonstrated. Thus when powdered gelatin of different pH is treated with nickel chloride and the salt not in combination is removed by washing with ice-cold water, the presence of nickel can be demonstrated in all gelatin solutions with a pH greater than 4·7 by using dimethylglyoxime as indicator.

Similar results were obtained with copper acetate, the gelatin retaining its blue colour, that is, the copper, with a pH greater than 4·7, and losing it if the pH is less than 4·7. With potassium ferrocyanide the gelatin retains the ferrocyanide on the acid side of the isoelectric point and loses it on the other side.

The behaviour of gelatin towards acid and basic dyes is interesting. A basic dye should be retained by the gelatin when it is functioning as an acid, that is, on the alkaline side of the isoelectric point where gelatin anions are present; on the acid side and at the isoelectric point the dye should readily be removed by washing. Experiments with the basic dye, neutral red, confirmed these predictions. Similarly, acid dyes, like acid fuchsin, combine with gelatin only when the pH is less than 4·7.

So far it has been shown that the hydrion concentration of a gelatin solution determines the amount of combination which occurs, but no proof has been given that chemical combination does actually take place; the ions may have been simply adsorbed. Such proof has been furnished as follows:—

It is known from ordinary physico-chemical measurements that nitric, oxalic and phosphoric acids each dissociate as monobasic acids at ordinary concentrations. It should follow, therefore, that if decinormal solutions of these acids are used, three times as many cc. of phosphoric and twice as many cc. of oxalic acid would be necessary to bring 100 cc. of a one per cent. solution of isoelectric gelatin to a given pH, e.g., 3·0, as would be required of nitric acid. On the other hand, sulphuric acid at such concentrations dissociates as a dibasic acid, so that the volume of sulphuric

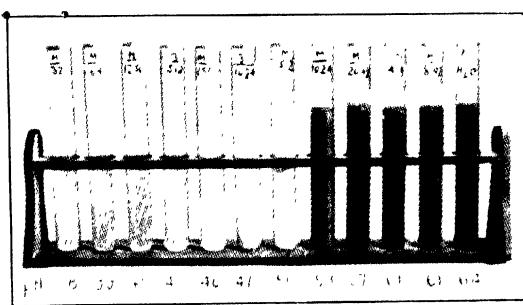


Fig. 2.



acid solution used should be the same as that of nitric acid solution of equivalent strength. The accompanying figure, Fig. 3, shows that the experimental results are in agreement with the theory, if allowance is made for experimental error.

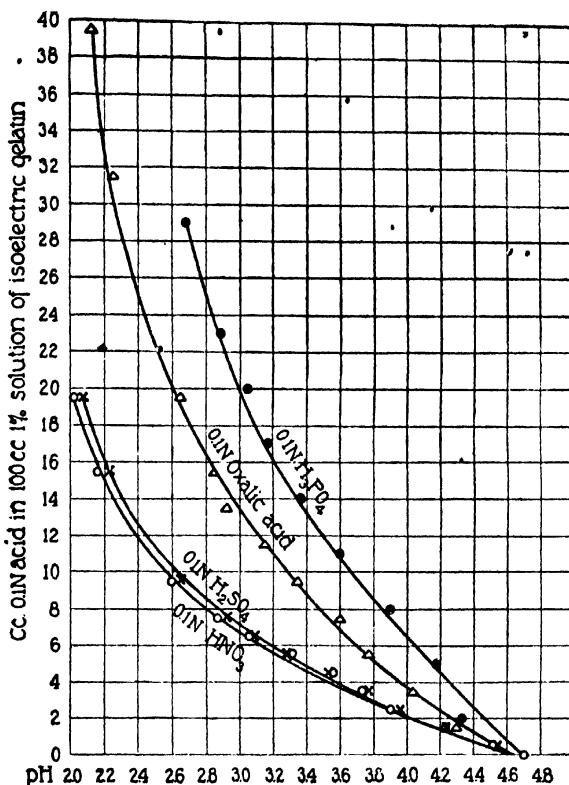


Fig. 3.

Exactly similar results were obtained with the hydroxides of lithium, sodium, potassium, calcium, and barium, taking into account that the first three are mono-acid bases and the last two dissociate as diacid bases.

Having established the fact that the combination between gelatin and acids and alkalis is of a purely chemical nature, it should be possible to predict the influence of acids and bases on the physical properties of gelatin. Thus, if the effects of the following acids, each of which dissociates into two ions at ordinary dilutions, on the swelling of gelatin are compared at the same pH, the amount of swelling should be the same:—hydrochloric, hydrobromic, nitric, phosphoric, acetic, monochloroacetic,

dichloroacetic, trichloroacetic, oxalic, tartaric and citric acids. The accompanying figure, Fig. 4, shows that theory and experiment agree,

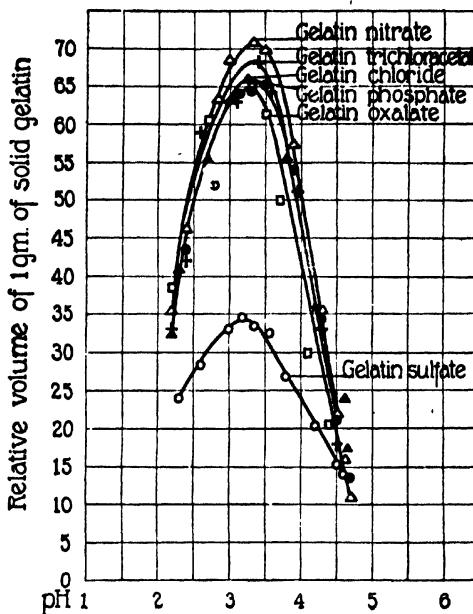


Fig. 4.

if one takes into account the large errors of experiment which occur in measurements of this kind. Similar results were obtained from the properties of viscosity and osmotic pressure, all of the curves showing a maximum at a pH of about 3.2.

Sulphuric acid dissociates in solution giving a divalent anion,  $\text{SO}_4^{2-}$ , and the figure shows that the values obtained are different from those with monovalent ions; the curve is of the same type, reaching a maximum at  $\text{pH} = 3.2$ , but the maximum attained is only about one-half of that given by the other acids.

Similarly it was found that the swelling curves for lithium, sodium, potassium and ammonium hydroxides are the same, as also those of calcium and barium hydroxides, but the latter are lower than the former.

It is obvious from the above that the results are not in agreement with the Hofmeister series, according to which the curves for phosphoric, oxalic, and citric acids should be in the same region as that for sulphuric acid, but not in the region of the hydrochloric acid curve. A true comparison of the action of different substances on gelatin can only be made when the pH of gelatin is taken into account.

In the *J. Gen. Physiol.* (1921, 3, 391), Loeb deals especially with the question of the Hofmeister series and the difference in the effect of acids, alkalis and salts on proteins. Attempts have been made by various

investigators to explain why the influence of neutral salts on proteins is different from that of acids and bases. Pauli (*Fortschr. naturwiss. Forschung*, 1912, **4**, 223) states that while acids and alkalis form salts with proteins, neutral salts form "adsorption compounds" with electrically neutral, that is, non-ionised protein molecules, both ions of the salt being simultaneously adsorbed by the protein molecule. The above results of Loeb indicate, however, that only one of the two ions of the neutral salt can combine at one time with the protein, and that at the isoelectric point no combination takes place.

Statements made to the effect that acids and alkalis increase, while salts depress the osmotic pressure of gelatin (Lillie, *Amer. J. Physiol.*, 1907-08, **20**, 127) cannot be considered to be true, since no account was taken of the pH of the solutions. Also, it is not correct to speak of antagonism between the effects of acids and salts; it would be just as correct to say that there is an antagonism between much acid and little acid, since the viscosity, swelling, etc., of gelatin increase to a maximum and then decrease as the concentration of the acid is increased.

Loeb is not in agreement with the idea of Pauli (see later) that the swelling, increase in viscosity, etc., of proteins is due to the hydration of the protein ions present in the solution, and puts forward a theory that when acids or alkalis are added to isoelectric gelatin both ions of the acid or alkali influence the physical properties of the gelatin but in opposite directions. When acid is added the hydrions increase, while the anions decrease the osmotic pressure and viscosity. With low concentrations of acid the augmenting action of the hydrion on these properties increases more rapidly with increasing concentration of the acid than the depressing action of the anion, while when the pH of the solution falls below 3.3 or 3.0 the reverse is the case. This causes a drop in the curves for osmotic pressure, viscosity and swelling below a pH of 3.0. In the case of alkalis the OH<sup>-</sup> ions increase the viscosity, etc., whilst the metal ions have a depressing effect.

When, however, a neutral salt is added to isoelectric gelatin there is no effect as long as the concentration does not reach the value required for precipitation. If the salt is added to gelatin solutions on either side of the isoelectric point, only a depressing action of that ion which has the opposite charge to that on the protein ion is observed.

#### *1 per cent. Gelatin Solution of pH=4.0.*

The addition of sodium chloride solutions in concentrations varying from M/8192 to M/8 causes a continuous decrease in the viscosity. The pH of the solution is not altered by the addition of sodium chloride (A pH of 4.0 is obtained by the acidification to the proper extent with hydrochloric acid, so that the solution is one of gelatin hydrochloride). If solutions of hydrochloric acid are used instead of sodium chloride, the viscosity rises to a maximum with M/256 hydrochloric acid (the pH of the solution is then about 3.0) and then falls as the concentration of the acid is further increased. Loeb interprets these results by saying that while the addition of hydrions increases the viscosity of a solution of gelatin hydrochloride of pH=4.0, the addition of sodium ions does not have such effect, but the chloride ion depresses the viscosity in both cases, no matter whether sodium chloride or hydrochloric acid is added to the gelatin solution; the depressing action of the chloride ions increases with its concentration.

#### *1 per cent. Gelatin Solution of pH=3.0.*

The addition of sodium chloride or hydrochloric acid in both cases causes a drop in the viscosity, the concentration where a drop becomes

very pronounced being with somewhat higher concentrations of hydrochloric acid than sodium chloride.

The above results are in accordance with Loeb's theory, but it is possible to give an explanation of them based on (1) Pauli's ideas of the hydration of protein ions as distinct from the non-hydration of the unionised protein molecule, and (2) on the suppression of the dissociation of a salt—in this case gelatin hydrochloride—by the addition to the solution of a salt with a common ion.

#### *1 per cent. Gelatin Solution in the Presence of Various Salts.*

It is found that the depressing effect on the viscosity of equimolecular solutions of the chlorides of sodium, calcium, and lanthanum, that is, of  $\text{NaCl}$ ,  $\text{CaCl}_2$ , and  $\text{LaCl}_3$ , is in exact (?) proportion to the concentration of the chloride ions in the solution, that is, as 1 : 2 : 3. [This, again, is what one would expect from the dissociation theory, but Loeb simply takes it as proof that such a depressing effect is due to the anions alone, since they are oppositely charged to the gelatin ion, the cation having no augmenting effect.]

Loeb further supports his idea that it is the anion which is responsible for lowering the viscosity by experiments with sodium chloride, sodium sulphate and sodium ferrocyanide, it being shown that the higher the valency of the anion the greater is the depressing effect on the viscosity, osmotic pressure and swelling. Apparently Loeb believes that such results cannot be explained by the dissociation theory. It must be remembered, however, that before the dissociation theory can be applied it is necessary to know the kind of equilibrium which is set up when, for example, sodium sulphate is added to a solution containing gelatin, gelatin hydrochloride and free hydrochloric acid. At present our knowledge with regard to such equilibria is practically nil.

#### *Influence of Alkalies.*

In analogy to the effect of acids it is shown that the addition of potassium hydroxide to sodium gelatinate of  $\text{pH}=12\cdot0$  depresses the viscosity in the same way and to the same extent as the addition of potassium chloride; the addition, however, of potassium hydroxide to sodium gelatinate of  $\text{pH}=4\cdot8-8\cdot0$  increases, whilst the addition of potassium chloride depresses, the viscosity. The depressing effect of salts on a metal gelatinate is due to the cation of the electrolyte added, that of bivalent cations being greater than that of monovalent cations, the valency of the anion having no effect.

In considering the question of salts and the Hofmeister series, it must be remembered that the addition of a salt to a gelatin solution will in many cases alter the pH of the solution; this alteration will be large in the case of certain salts, whereas in the case of others, e.g., sodium chloride, it may be non-existent or very small. For a true comparison to be made between the action of various salts it is therefore necessary to compare them in solutions of the same pH.

The following figure, Fig. 5, represents the depression of viscosity, in arbitrary units, of a gelatin chloride solution of  $\text{pH}=5\cdot0$ , by different concentrations of salts with univalent anions, namely sodium chloride, sodium dihydrogen phosphate, sodium hydrogen tartrate, sodium dihydrogen citrate and sodium acetate. The curve for sodium sulphate is added for comparison to show the behaviour of a salt with a bivalent anion. (The monosodium salts of weaker dibasic and tribasic acids

dissociate into a sodium ion and a monovalent anion, e.g.,  $\text{H}_2\text{PO}_4^-$  from  $\text{NaH}_2\text{PO}_4$ .)

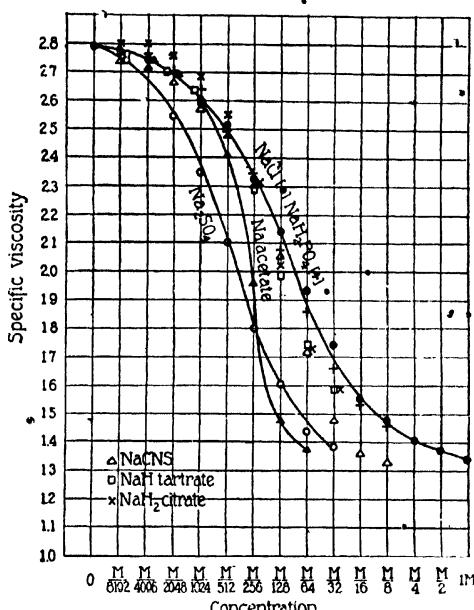


FIG. 5.

Loeb's valency rule demands that the relative depressing effect of these salts, with the exception of sodium sulphate, should be the same. This is apparently only true for sodium chloride and sodium dihydrogen phosphate, the other salts acting in accordance with the usual Hofmeister series. This may possibly be due to the added salts altering the pH of the solution, and the following table, which gives the results of measurements of the pH of these various solutions proves that this is the case.

## MOLECULAR CONCENTRATION OF SALTS USED

|                               | M   | M    | M    | M    | M    | M   | M   | M   | M    | M    | M    | M    | M    |
|-------------------------------|-----|------|------|------|------|-----|-----|-----|------|------|------|------|------|
|                               | 0   | 8192 | 4096 | 2048 | 1024 | 512 | 256 | 128 | 64   | 32   | 16   | 8    | 4    |
| $\text{NaCl}$ ...             | 3.0 | 3.0  | 3.0  | 3.0  | 3.0  | 3.0 | 3.0 | 3.0 | 3.0  | 3.0  | 3.0  | 3.0  | 3.0  |
| $\text{Na}_2\text{SO}_4$ ...  | 3.0 | 3.0  | 3.0  | 3.0  | 3.0  | 3.0 | 3.0 | 3.0 | 3.05 | 3.1  | 3.2  | 3.3  | 3.7  |
| $\text{NaH}_2\text{PO}_4$ ... | 3.0 | 3.0  | 3.0  | 3.0  | 3.0  | 3.0 | 3.0 | 3.1 | 3.2  | 3.3  | 3.4  | 3.45 | 3.5  |
| $\text{NaCNS}$ ...            | 3.6 | 3.0  | 3.0  | 3.0  | 3.0  | 3.0 | 3.1 | 3.2 | 3.3  | 3.6  | 3.9  | 4.2  | 4.4  |
| $\text{NaH}$ tartrate ...     | 3.0 | 3.0  | 3.0  | 3.0  | 3.0  | 3.0 | 3.1 | 3.2 | 3.3  | 3.45 | 3.5  | 3.55 | 3.6  |
| $\text{NaH}_2$ citrate ...    | 3.0 | 3.0  | 3.0  | 3.0  | 3.0  | 3.1 | 3.2 | 3.4 | 3.6  | 3.7  | 3.75 | 3.8  | 3.85 |
| $\text{Na}$ acetate ...       | 3.0 | 3.0  | 3.0  | 3.05 | 3.1  | 3.3 | 3.7 | 4.3 | 4.6  | —    | —    | —    | —    |

Thus, neglecting sodium sulphate, as long as the pH of the various solutions remains equal to 3.0 their viscosities agree; variation in the pH corresponds with an alteration in the viscosity. That it is this alteration in the pH which causes the abnormal behaviour, was further proved by the fact that if gelatin solutions containing sodium acetate in various concentrations are so made, by the addition of acetic acid,

that the pH remains the same, the curves for sodium chloride and sodium acetate are the same within the error of experiment. See Fig. 6.

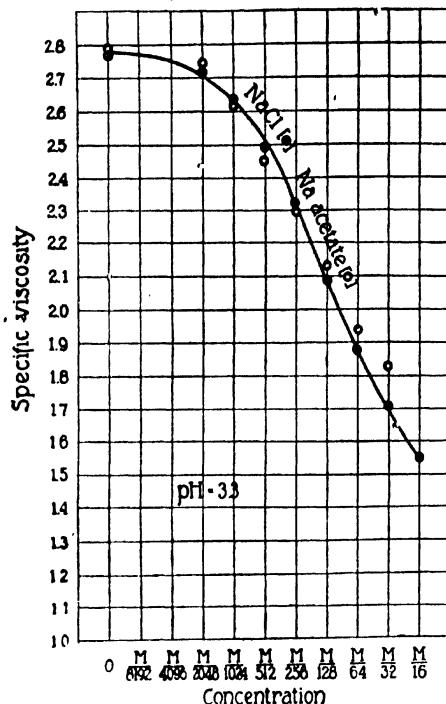


FIG. 6.

(Sodium acetate was chosen because it shows the most abnormal behaviour.) Thus the statement that is often made that sodium acetate acts like sodium sulphate, which is interpreted to mean that the univalent acetate anion acts like the bivalent sulphate anion and not like the univalent chloride or nitrate anion, is incorrect; the anomalous effect ascribed to the acetate anion in colloidal literature is in reality due to the depression of the hydrogen ion concentration of the gelatin solution by the sodium acetate.

Experiments on swelling gave the same results.

Salts like disodium oxalate and disodium tartrate, which dissociate in solution giving two sodium ions and one bivalent anion, should, according to Loeb's theory, act in the same way as sodium sulphate, that is, equivalent solutions should give the same lowering of the viscosity as long as the pH is the same. This is found to be the case. Also, the osmotic pressure, viscosity and swelling of sodium gelatinate should be depressed by the cation of a salt, and the more so the higher the valency of the cation. This was found to be true for the swelling of sodium gelatinate of pH=9.3, the depressing effect of sodium chloride being half as great as that of sodium sulphate of equimolecular concentration, while that of calcium chloride is considerably greater owing to the fact that calcium is bivalent.

In the experiments quoted in the above paper the concentrations of the salt used were in most cases not greater than M/16. Now at concentrations of salts which are in the neighbourhood of M/16 the values of the properties of viscosity, swelling, etc., of gelatin are near the minimum characteristic of the isoelectric point, and it therefore follows that it is not safe to draw conclusions concerning specific effects of ions on the swelling, etc., of gelatin when the concentration of electrolytes in solution exceeds M/16.

In two later papers (*J. Gen. Physiol.*, 1921, **3**, 557, 667) Loeb measures the potential difference which exists under equilibrium conditions between a solution of gelatin hydrochloride contained in a collodion bag and an outside aqueous solution (without gelatin); the depressing effect of neutral salts on the potential difference was also determined. It is found that the depression of the potential difference by the salt can be calculated fairly accurately on the basis of Nernst's theory (logarithmic formula) and the assumption that the potential difference which exists at the point of equilibrium is due to the difference between the hydron concentration on the opposite sides of the membrane. Since the difference in concentration of the hydron on both sides of the membrane is due to the Donnan equilibrium (see later: Procter's investigations), it can be readily deduced that the difference in potential should also be calculable from the difference in the concentration of the chloride ions on the two sides of the membrane, and this is found to be the case.

It is shown that the influence of the hydron concentration on the potential difference of a gelatin hydrochloride solution is similar to the effect it has on the osmotic pressure, swelling and viscosity of gelatin solutions. Because of this similarity it should follow that since the Donnan equilibrium determines the potential difference it should also determine the osmotic pressure, viscosity and swelling. Loeb endeavours to bring evidence in support of this for osmotic pressure in *J. Gen. Physiol.*, 1921, **3**, 691, and for viscosity in *ibid.*, 1921, **3**, 827; 1921, **4**, 73, 97. In the papers on viscosity it is proved that the viscosity of suspensions of powdered gelatin in water is influenced by electrolytes in the same way as the viscosity of solutions of gelatin.

Attention may also be called to the following papers, which refer indirectly to the amphoteric character of gelatin: *J. Gen. Physiol.*, 1920, **2**, 255, 273; *Science*, 1921, **53**, 77. Other results obtained by Loeb will be referred to in the later sections.

#### PAULI'S INVESTIGATIONS

Pauli and his co-workers have published a large number of papers on proteins in *Pfluger's Archiv*, in the *Kolloid Zeitschrift*, and in the *Biochemische Zeitschrift*, the first paper, which was published under the name of Paschelos (Pauli's original name) being published in 1897. Recently Pauli has published Part I. of his "*Kolloidchemie der Eiweißkörper*," which contains a summary and a discussion of a large portion of his work, although a considerable amount remains to be dealt with in succeeding parts.

Pauli's publications deal not only with gelatin, but to a very large extent with other proteins (various sera, globulins, casein, etc.) which behave analogously to gelatin in many respects; observations on gelatin are scattered through the various papers.

For the first 10 years or so the effects of various salts, acids and bases on the swelling, viscosity, etc., of gelatin were investigated without paying particular attention to its amphoteric nature, but in the more recent work the exact methods of physical chemistry have been applied towards a solution of the various problems. These later results will be dealt with first, since the earlier results are best discussed in the light of later observations. Many of the statements made in what is to follow

will be understood from what has already been said in connection with Loeb's investigations, so that detailed explanations will not be necessary.

Throughout his work Pauli makes the assumption that when gelatin (or other protein) combines with electrolytes added to the solution, the gelatin salt formed dissociates, and the ions of gelatin which result are hydrated. It is the hydration of the gelatin ions which gives rise to the increase in viscosity, etc. Since the dissociation of gelatin as an amphoteric electrolyte is a minimum at the isoelectric point the viscosity should also be a minimum there, which corresponds with the facts. As acid, for example, hydrochloric acid, is added in increasing quantities to the solution, the amount of gelatin hydrochloride, and therefore of gelatin ions will increase, so that the viscosity will increase as the pH diminishes. All the acid, however, does not combine with the gelatin, and after a time the amount of uncombined hydrochloric acid is such that it begins to throw back the dissociation of the gelatin hydrochloride. Consequently there should be a maximum amount of dissociation of the gelatin salt and a corresponding maximum in the viscosity, which is in accordance with the experimental results.

#### *Isoelectric Gelatin.*

Soon after Michaelis and Grimeff (*Biochem. Zeitschr.*, 1912, **41**, 373) had proved that the isoelectric point of gelatin was at pH = 4.7, Pauli, with Matula and Samec (*Kolloidchemie der Eiweißkörper*, p. 32; also *Kolloid Zeitschr.*, 1913, **12**, 222), showed that when the necessary hydron concentration in the solution was obtained by the addition of a mixture of sodium acetate and acetic acid, viscosity and swelling were a minimum at the isoelectric point, whilst the precipitability with alcohol was most pronounced. On either side of the isoelectric point, the viscosity and swelling increased with alteration in the pH, the increase being the more rapid as the pH diminished, that is, as the solution became more acid.

It can be shown theoretically, that at the isoelectric point the dissociation of an amphoteric electrolyte (mono-acid base and mono-basic acid) is independent of the dilution, that is, that the hydron concentration is independent of the concentration of the solution. It should follow, therefore, that the minimum viscosity and swelling should be, at the same pH, independent of the concentration of the solution as regards gelatin. This was found to be the case when the solution was made isoelectric by means of the sodium acetate-acetic acid mixture (Pauli and Matula, *Kolloid Zeitschr.*, 1913, **12**, 222) but with sulphuric acid the point of minimum viscosity was found to alter in the direction of increasing concentration of the acid as the gelatin concentration of the solution increased (Pauli and Samec, *Kolloidchemie der Eiweißkörper*, p. 35). In the case of serum albumin it was found that the concentrations at which minimum viscosity occurred varied with the acid used, the acids being hydrochloric, sulphuric and oxalic. Since the minimum viscosity, etc., should correspond, according to Pauli, with the maximum number of neutral particles of the protein in solution, Pauli draws the conclusion that this maximum number does not occur at the isoelectric point in the presence of strong acids. Other evidence is adduced in support of this conclusion, and it would follow that gelatin does not behave in exactly the same way towards strong and weak acids, showing a higher basicity towards the former than the latter. Such a conclusion does not agree with Loeb's results. As far as the viscosity measurements (relative to that of water) of Pauli are concerned, an accuracy to the third place of decimals is assumed, and the conclusions drawn from the results are only valid if this assumption holds. It is very doubtful, however, whether the viscosities of such substances as gelatin can be measured so accurately. Moreover it is difficult to see why, in such low concentrations of acid as are necessary to give isoelectric gelatin, strong acids should behave differently from weak acids (compare Wintgen and Krüger, *Kolloid*

*Zeitschr.*, 1921, **28**, 81; Wintgen and Vogel, *ibid*, 1922, **30**, 45. These papers also give the following references to earlier literature on the behaviour of gelatin towards acids:—Hoffmann, *Zentrbl. f. klin. Medizin*, 1889, p. 773; 1890, p. 521; Cohnheim, *Zeitschr. f. Biol.*, 1896, **33**, 489; Guttentag, *Muenchener med. Wochenschrift*, 1896, **43**, 147.

#### Gelatin Salts with Acids.

In these experiments Pauli made use of electrode potential measurements in order to determine, when hydrochloric acid, for example, was used, what proportion of the hydriions and of the chloride ions was bound by the gelatin.

Using such methods, Bugarsky and Liebermann (*Archiv. Physiol.*, 1898, **72**, 51) had deduced that the compound formed between protein

and hydrochloric acid, e.g.,  $\text{G} \begin{array}{c} \text{NH}_3\text{Cl} \\ \diagdown \\ \text{COOH} \end{array}$  for gelatin, was very little

dissociated. This was disputed by Brailsford Robertson and definitely shown to be incorrect by Manabe and Matula (*Biochem. Zeitschr.*, 1913, **52**, 369); compare also Chiara (*ibid.*, 1911, **33**, 167). Their results are best indicated in the following diagram, Fig. 7, for a 1 per cent. solution of gelatin. The ordinates represent the concentration of the "bound" ions and the abscissae the concentrations of the acid used.

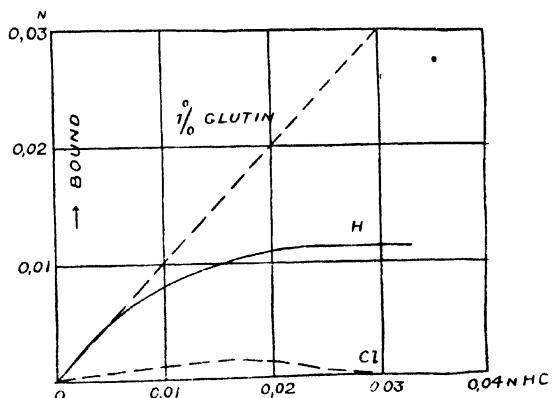
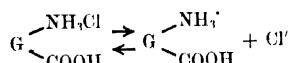


FIG. 7.

If the hydriions and chloride ions are completely bound by the gelatin the curves for both should coincide with the dotted curve. The figure shows, however, that the chloride ions remain practically free at all the concentrations investigated (up to 0.05 N-HCl); the hydriions are completely bound up to a concentration of about 0.005N, above which concentration the proportion bound becomes less, ultimately approaching a maximum quantity independent of the concentration of the acid. The results therefore give a definite proof that a salt is formed which is almost completely dissociated in accordance with the scheme:—



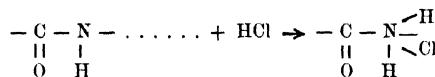
excess of acid up to 0.05N HCl not appreciably diminishing the dissociation of the gelatin chloride.

In the case of serum albumin (1.09 per cent.) it is found that excess of acid above 0.02N begins to suppress the dissociation of the chloride formed; there is thus a maximum degree of dissociation, which occurs at a definite concentration of hydrion, and if the viscosity depends on the dissociation, that is, on the concentration of protein ions, it also should be a maximum at this point. This is found to be the case with horse serum. Similarly the precipitability with alcohol should go through a maximum which again is found to be true. (Schorr. *Biochem. Zeitschr.*, 1911, **37**, 424.)

In the case of gelatin, however\*, the dissociation of the gelatin chloride does not go through a maximum, at all events up to a concentration of 0.05N HCl, but nevertheless the viscosity shows a maximum. This maximum occurs in the presence of 0.005N hydrochloric acid with a 0.3 per cent. gelatin at 30°, 35° or 40°, and with a 0.1N hydrochloric acid for a 1 per cent. solution of gelatin at 35° (Pauli and Falek, *Biochem. Zeitschr.*, 1912, **47**, 270). For gelatin, therefore, the connection between viscosity and dissociation does not seem to hold. Since, however, the connection holds for other proteins, it is reasonable to suppose that it is also true for gelatin, and that the discrepancy may be due to some error in the determination of the chloride ion in the case of gelatin solutions.

Measurements with other acids (sulphuric, acetic, trichloroacetic, citric, and oxalic acids, etc.) have been made (Pauli and Hirschfeld, *Biochem. Zeitschr.*, 1914, **62**, 245; Pauli and Handovsky, *ibid.*, 1909, **18**, 340), but in these cases there is so far no satisfactory method of determining the concentration of the anions, so that it is difficult to draw quite satisfactory conclusions from the results. The matter is also all the more complicated by the occurrence of considerable hydrolysis, but, in general, it may be stated that the results are in accordance with the amphoteric character of the proteins.

An interesting paper from Pauli's laboratory (Blasel and Matula, *Biochem. Zeitschr.*, 1914, **58**, 417), is in connection with deaminogelatin, which is obtained from gelatin by treatment with nitrous acid, the terminal-NH<sub>2</sub> groups being thereby removed. If the basic character of a gelatin is due to these terminal-NH<sub>2</sub> groups, their removal should give a substance which is non-basic in character. It is found, however, that it still possesses a distinct combining capacity for hydrochloric acid, although less than in the case of gelatin itself. This indicates that the internal peptide linkages can function as acid groups, in accordance with the scheme:—



In direct contradiction to gelatin, however, this combination with acid does not lead to any increase in the viscosity of the deaminogelatin. The salt formed is dissociated, and it therefore follows, according to Pauli's theory, that the ions formed on dissociation are not hydrated, as they are when the salt is formed by combination of acid with the terminal  $-NH_2$  groups. Deaminogelatin does resemble other proteins, however, in that the point of maximum difference between the bound hydrogen and chloride ions corresponds with the minimal precipitability by phenol.

### *Gelatin Salts with Bases.*

The combination of proteins with the bases can only be determined electrometrically by measurement of the hydron concentration of the solutions. The concentration of the hydrons gives a measure of the hydroxyl ion concentration, since in all solutions the relation  $[H^+] \cdot [OH^-] = \text{const} (= 10^{-14} \text{ at } 25^\circ)$  must hold. The results obtained (Handwyk, *Biochem. Zeitschr.*, 1910, **25**, 510; Chiari, *ibid.*, 1911, **33**, 167; Schorr,

*ibid.*, 1911, **37**, 424; Pauli and Falek, *ibid.*, 1912, **47**, 270; Wagner, *ibid.*, 1921, **104**, 190) admit of a similar interpretation to that given for the acid

salts, namely that the salt formed, e.g.,  $\text{G}-\text{NH}_3\text{OH}$  dissociates in solution  
 $\text{COONa}$

e.g., into  $\text{G}-\text{NH}_3\text{OH}$  +  $\text{Na}^+$ , and that corresponding with this dissociation  
 $\text{COO}^-$

which is accompanied by hydration of the gelatin ion, there is an alteration in the viscosity and precipitability by alcohol. With increasing concentration of alkali there will be a point at which the dissociation is a maximum, since at higher concentrations the dissociation of the gelatinate will begin to be suppressed. Corresponding with this maximum dissociation there is a maximum viscosity and a minimum precipitability with alcohol.

#### *Gelatin, Acetic Acid, and Ammonium Hydroxide.*

Under ordinary circumstances the dissociation of a weak acid like acetic acid or a weak base such as ammonium hydroxide, is so small that in order to make comparisons with strong acids or bases it is necessary to have a very dilute solution of the strong acid or base, and a strong solution of the weak acid or base, if solutions of the same ionic concentration are to be obtained. For example, a 0.002N solution of hydrochloric acid has the same hydron concentration as a 0.2N solution of acetic acid. With such strong solutions of acetic acid it is possible that effects other than the mere combination of the gelatin, as a base, with the acid plays a part. This is found to be the case, the results obtained with acetic acid (Pauli and Hirschfeld, *Biochem. Zeitschr.*, 1914, **62**, 245; Loeb, *J. Gen. Physiol.*, 1920, **3**, 85, 247) being abnormal; albumin, for example, apparently combines with four times as much acetic acid (0.2N) as it does with hydrochloric acid (0.002N). Similar abnormal results have been found with ammonium hydroxide as compared with sodium hydroxide (Pauli and Spitzer, *Kolloidchemie der Eiweisskörper*, p. 77).

#### *Combination of Gelatin with Salts.*

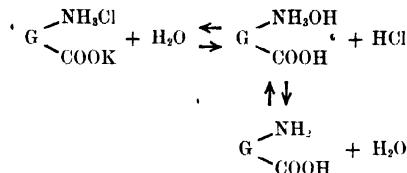
The salts so far investigated have been potassium chloride (Pauli and Oryng, *Biochem. Zeitschr.*, 1910, **70**, 368) and silver nitrate (Pauli and Matula, *ibid.*, 1917, **80**, 187). The gelatin used was dialysed against distilled water for several weeks at 10° so that presumably it was on the alkaline side of the isolectric point although measurements of the pH of the gelatin and of the various solutions were apparently not made. The results do not admit of such a clear interpretation as do those obtained by Loeb, nor are they in complete agreement with the same.

#### *Potassium Chloride.*

Electrometric measurement had to be carried out by means of a mercury electrode containing mercurous chloride; this introduced various complications which had to be corrected for, and it is not certain to what extent these corrections are satisfactory.

Quantitatively, using potassium chloride varying in concentration from 0.1 to 0.001N, the results showed that chlorine is taken up by the protein to a very large extent at the lower concentrations of the chloride, but that the combination decreases relatively with increasing concentration, approaching a limiting value which, for 1 per cent. gelatin is approximately at 0.05N potassium chloride solution. It was further found that in the presence of potassium chloride (the concentrations are not stated), the direction of wandering of the gelatin to the electrode (it wanders to the anode) was not altered.

Pauli assumes that the potassium chloride can combine with the gelatin giving a salt such as  $\text{G}-\begin{matrix} \text{NH}_3\text{Cl} \\ \diagdown \\ \text{COOK} \end{matrix}$  which may then undergo hydrolysis according to the scheme:—



It may also undergo ordinary dissociation. From the cataphoresis (electrical wandering) experiments and from the fact that the viscosity of the solutions is not much altered by the addition of potassium chloride, Pauli and Oryng draw the conclusion that the ions of the gelatin salt formed are not strongly hydrated and that anion and cation are present to the same extent.

The above conclusions are, of course, quite at variance with Loeb's ideas. Actually, however, if Loeb's theories are assumed to be correct, the results of Pauli and Oryng can be explained as follows.—on the alkaline side of the isoelectric point the alteration in viscosity with increase in the pH is relatively small compared with the alteration which takes place with diminishing pH on the acid side (*compare* Loeb, *J. Gen. Physiol.*, 1920, **3**, 85, 247; 1921, **3**, 391). Assuming that the gelatin had a pH of 7, its viscosity would not be much greater than gelatin with pH 4·7, and therefore addition of potassium chloride would have very little effect, since Loeb has shown (*J. Gen. Physiol.*, 1921, **3**, 402) that the addition of potassium chloride to sodium gelatinate of pH 4·8–8·0 always depresses the viscosity. Also, since the gelatin would remain on the alkaline side of the isoelectric point the addition of potassium chloride would not alter the sign of the cataphoresis.

Pauli and Oryng also found that the addition of sulphuric acid to the potassium chloride solution decreases the amount of combined chlorine, especially at low concentrations of potassium chloride. This is in accordance with Loeb's results, since the sulphuric acid would alter the gelatin to the acid side of the isoelectric point and it would dissociate as gelatin hydrochloride, giving chloride ions.

#### *Silver Nitrate.*

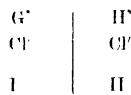
Using solutions of silver nitrate, varying in concentration from 0·0025–0·05N, in 1 per cent. gelatin, Pauli and Matula found, by electrometric measurements, that silver combined with the gelatin, that is, silver ions disappeared from the solution. Corresponding with this there was a diminution in the electrical conductivity of the solution. Measurements with varying concentrations of gelatin (up to 3·4 per cent.) showed that the amount of silver combined is not directly proportional to the concentration of the gelatin; the deviation from proportionality is much greater in dilute than in concentrated solutions, indicating greater hydrolysis of the silver salt compound in the dilute solutions. The direction of electrical wandering of the gelatin was not altered by the addition of silver nitrate, nor was the viscosity of the solutions appreciably changed. Pauli and Matula give similar explanations to those given for potassium chloride, but the results are readily explicable in accordance with Loeb's ideas. There is, however, one difficulty, in that it is stated that prolonged dialysis removed all the silver, giving a gelatin with the original properties. According to Loeb, this could only be the case if the gelatin were at, or on the acid side of, the isoelectric point.

In the presence of sodium nitrate or barium nitrate the silver is forced out of its combination with the gelatin to a greater or lesser extent.

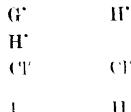
#### PROCTER'S INVESTIGATIONS

Professor H. R. Procter, in connection with his work on tanning, has carried out numerous investigations on the swelling of gelatin and of the behaviour of gelatin towards acids. To a very large extent the work seems to have been quite independent of that of other investigators, comparatively few references being made to their results. Procter has been ably assisted in his investigations by J. A. Wilson, and the chief papers published are as follows: Procter (*Kolloid Beihete*, 1911, **2**, 243), Procter (*Trans. Chem. Soc.*, 1914, **105**, 313) Procter and Wilson (*Trans. Chem. Soc.*, 1916, **109**, 307), Wilson (*J. Amer. Chem. Soc.*, 1916, **38**, 1982), Procter and Burton (*J. Soc. Chem. Ind.*, 1916, **35**, 404), Wilson and Wilson (*J. Amer. Chem. Soc.*, 1918, **40**, 886), Wilson (*3rd British Association Report on Colloid Chemistry*, 1920, p. 51). Other papers have been published in the *Jour. Soc. Leather Trades' Chemists* and in the *Jour. Amer. Leather Chemists' Association*, but these, for the most part, have repeated the accounts given in the above-mentioned papers.

Procter's theories are founded on what is known as the Donnan equilibrium (*Zeitschr. Elektrochem.*, 1911, **17**, 572; see also *System of Physical Chemistry*, Lewis, Vol. II., p. 275), which may be explained thus - Consider the salt gelatin hydrochloride, which may be written  $\text{GCl}$ , dissolved in water, the solution being in contact with a membrane (denoted by a vertical line), which is impermeable to the gelatin cation,  $\text{G}^+$ , and also to the undissociated molecules  $\text{GCl}$ , but will allow chloride ions,  $\text{Cl}^-$ , and any other ions to pass through it freely. Suppose that on the other side of the membrane there is an aqueous solution of hydrochloric acid,  $\text{HCl}$ . The initial state of things will be represented by :-



The  $\text{HCl}$  will, however, begin to diffuse from II to I until an equilibrium state is reached, represented by :-



It can be shown by thermodynamical reasoning that when the state of equilibrium is reached, the relation must hold that

$$[\text{H}^+]_{\text{II}} \times [\text{Cl}^-]_{\text{II}} = [\text{H}^+]_{\text{I}} \times [\text{Cl}^-]_{\text{I}}$$

where the square brackets represent concentrations. It can further be shown that the greater the initial concentration ratio of gelatin hydrochloride to hydrochloric acid, the less hydrochloric acid will diffuse through the membrane; that is, although the membrane is quite permeable to hydrochloric acid, the presence of the non-dialysing gelatin hydrochloride,  $\text{GCl}$ , in sufficient concentration on the one side is able to make the permeability of the membrane for hydrochloric acid in one direction almost vanish. In accordance with this Procter found that the concentration of free hydrochloric acid is always less in the solution absorbed by the gelatin than in the remaining external solution. This has been confirmed by Loeb (*J. Gen. Physiol.*, 1921, **3**, 557, 667, 691) by measurements on solutions of gelatin hydrochloride separated from an aqueous solution of hydrochloric acid by means of a collodion membrane; the pH of

the inside solution was always greater than that of the outside solution, and the difference in electric potential which existed between the two solutions could be calculated from the difference in pH by means of Nernst's logarithmic formula.

Procter further found that the sum of the amounts of free acid in both the gelatin and aqueous acid solutions is less than the amount in the solution before the introduction of the gelatin. This difference he attributes to chemical combination between the gelatin and some of the acid and regards the product as a hydrolysable but highly ionisable hydrochloride of gelatin. (This agrees with the electrometric measurements of Manabe and Matula.)

Procter's theory may be summed up as follows:—When gelatin is immersed in a dilute solution of an acid, e.g., hydrochloric acid, combination takes place between the gelatin molecules and the hydrons, resulting in the formation of highly ionised gelatin hydrochloride. The gelatin cation is incapable of diffusing into the outer liquid; the chloride anion ( $\text{Cl}^-$ ) will tend to diffuse, but is prevented from so doing because of the electrical attraction of the gelatin cation. The tendency of the chloride anion to diffuse out exerts on the jelly mass an outward pull which, being uniform in all directions, produces an increase in the volume of the jelly proportional to the magnitude of the pull.

Suppose that

$$\text{In the outer liquid, concentration of } [\text{H}^+] = [\text{Cl}^-] = x \text{ "}$$

$$\text{And in the jelly } \left\{ \begin{array}{l} " " [\text{H}^+] = y \\ " " \text{ Gelatin ions} = [\text{G}^+] = z. \end{array} \right.$$

Since, in the jelly, the total concentration of the positive ions must be equal to that of the negative ions (otherwise the jelly would be electrically charged), it follows that (in the jelly) the concentration of the chloride anions,  $\text{Cl}^-$ , =  $y + z$ .

The conditions of the Donnan equilibrium therefore will give

$$x^2 = y(y + z) \dots \dots \dots (1)$$

and it therefore follows mathematically that

$$2x < 2y + z,$$

that is, the concentration of the ions in the external solution is less than in the gel. The osmotic pressure in the gel will therefore be greater than in the external solution, and consequently swelling will occur until the forces of cohesion in the gel can establish equilibrium.

If  $e$  = excess concentration of diffusible ions in the gel then

$$2x + e = 2y + z \dots \dots \dots (2)$$

and the swelling should be proportional to the value of  $e$ .

It is possible, by means of electrometric measurements, to determine experimentally the values of  $x$ ,  $y$  and  $z$ , and hence to calculate the values of  $e$  for different solutions. It is found that as the pH of the external solution decreases from 3.75 to 1.19, that is, as the acidity increases, the value of  $e$  rises to a maximum and then falls, the maximum being at pH = 2.4 (approx.). The rise and fall of  $e$  is in accordance with theory, since if equations (1) and (2) are solved for  $e$ ,  $y$  being eliminated,

$$e = -2x + \sqrt{4x^2 + z^2}$$

and therefore, as  $x$  increases from zero  $e$  must increase to a maximum and then decrease, since  $z$  has a limiting value conditioned by the combining power of the gelatin. Also, it is known that the swelling of gelatin, with decreasing pH, increases from a minimum at the isolectric point, rises to a maximum and then decreases again. If the two curves,  $e$ —pH, and swelling—pH, are plotted they both show a maximum at the same pH = 2.4 (Atkin, *J. Soc. Leather Trades' Chemists*, 1920, 4, 248). Loeb found the maximum swelling at pH = 3.0, but the washing

with water according to his method may have hydrolysed some of the gelatin hydrochloride which had been formed, thus tending to form a lower hydron concentration or an increased pH value.

In the case of acetic acid, which is only slightly dissociated, a greater total concentration of the acid is required to produce nearly complete combination of the gelatin with the acid, because the degree of combination is determined by the value of  $y$ , which even in the more concentrated solutions will be small because of the repression of the ionisation of the acid by the highly ionisable gelatin salt. For this reason the swelling of gelatin in acetic acid increases with increasing total concentration of the acid and is not repressed by addition of excess; in fact the swelling continues up to a strength of acid of N/1, beyond which, solution of the gelatin takes place. Even in this case, however, Atkin (*loc. cit.*) shows that the swelling is probably at a maximum in the normal solution, the pH being approximately 2·4. The somewhat stronger formic acid shows a maximum swelling at pH = 2·4 (Atkin) whilst the very weak acids such as boric acid, produce little, if any, swelling.

The repression of the swelling of gelatin by the addition of a salt of the acid, which is highly dissociated, is caused by the apparent increase of  $x$  produced, which results in a diminution of the value of  $e$  and consequently in a diminution of the swelling, this action being assisted to some extent by the repression of the ionisation of the salt of the gelatin. Wilson and Wilson (*J. Amer. Chem. Soc.*, 1918, **40**, 886) have extended Procter's theory to the case of the action of any electrolyte (acid, salt or base) on gelatin or any other colloid.

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### VISCOSITY OF GELATIN

The viscosity of a liquid is the resistance offered to shearing, to stirring, or to flow through a capillary tube. The viscosity coefficient is the force required to move at unit velocity a plate of unit surface separated from another plate of the same size by a layer of unit thickness. Based on this definition the formula may be derived which gives the connection between the viscosity  $\eta$ , and the volume  $v$  of a liquid which, driven by a pressure  $p$ , flows through a tube of length  $l$  and radius  $r$  in the time  $t$ , namely  $\eta = \frac{\pi r^4 pt}{8l v}$  (Poiseuille's equation).

For this equation to be valid it is a necessary condition that the pressure  $p$  shall be just sufficient to drive the liquid through the tube, that is, the liquid shall have zero velocity as it leaves the tube. In actual practice this condition cannot, of course, be fulfilled, but any correcting factor to the above formula can, by the use of long and narrow capillary tubes, be made so small that it may be neglected. As a matter of fact it has recently been shown (Griffiths and Griffiths, *Proc. Phys. Soc.*, 1921, **33**, 231) that the coefficient of viscosity of water flowing through capillary tubes of 1.5-2.0 mm. diameter at rates of one litre in 2.24 years is the same as with ordinary rates of flow.

Thus the first essential of an apparatus for the measurement of the viscosity of liquids is a long and fine capillary tube. As used for ordinary liquids and solutions, the Ostwald apparatus, which is really a modified Poiseuille apparatus, is used, in which the capillary tube is about 0.25-0.4 mm. in diameter and 10-12 cm. long. The bulb, above and below which are the marks defining the volume of liquid which should flow through the capillary, is of such dimensions that the time of flow is at least 100 seconds, using water as the liquid.

Capillary tubes of the dimensions given are satisfactory for liquids and solutions which have viscosities less than, or not very much greater than, that of water, but for such viscous solutions as those of gelatin the time would be inordinately long. It is therefore necessary to have wider tubes, or else to apply a head of pressure to the tube to force the liquid through in a reasonable time. The use of a wide tube is apt to give rise to erroneous results, since when the same tube is used for water, as the standard substance of comparison, the velocity of flow of the water through the tube is so great that turbulent flow results. Bingham (*Amer. Chem. J.*, 1905, **34**, 481) was apparently one of the first to use a head of pressure, and the method has since been repeatedly used by him (References may be made, among other papers, to the following: *J. Ind. Eng. Chem.*, 1914, **6**, 233; *Bull. Bur. Standards, U.S.A.*, 1918, **14**, (T), 59; *Proc. Am. Soc. Testing Materials*, 1918, **18**, 373). Ubbelohde (Compare *Z. Elektrochem.*, 1913, **19**, 32) has also designed a similar apparatus. The convenience of such apparatus is, that when the head of pressure is so great that the hydrostatic pressure of the column of liquid in the apparatus can be neglected, the time of flow of a liquid through the viscometer is directly proportional to the viscosity, the head of pressure being constant. If the viscometer is calibrated with a standard liquid it is therefore not necessary to determine the density of the liquids used, as would be the case for ordinary viscometers.

If varying heads of pressure are used in different experiments, the viscosity,  $\eta$ , is given by the relation

$$\eta = k pt$$

where  $k$  is a constant and  $p$  and  $t$  are the pressure and time respectively. It follows that for the same liquid the volume of the product  $pt$  must be constant.

The importance of the constancy of the product  $\rho t$  in the case of gelatin solutions was not realised until the work of Rothlin in 1919 (*Biochem. Zeitschr.*, 1919, **98**, 34; compare also Hess, *Kolloid Zeitschr.*, 1920, **27**, 254), although previous to that date numerous measurements on viscosity had been made. Rothlin made use of a viscometer designed by Hess (*Münch. med. Wochenschr.*, 1907, **45**, No. 32, 1590) which consists in principle of two horizontal capillary tubes connected with a common pressure bulb. The same pressure is applied to both the tubes for the same time and the volumes of water and of the liquid under investigation flowing through the respective tubes are measured; since the volume flowing through is inversely proportional to the viscosity of the liquid, the unknown viscosity can be determined, knowing that of water.

In the case of a 50 per cent. solution of glycerol, for example, Rothlin found that with pressures varying between 39.7 and 0.95 mm. of mercury (there were 7 intermediate pressures) the time of flow through the viscometer varied from 2.9 to 120.0 seconds, but the product  $\rho t$  remained constant within the error of experiment.

In the case of gelatin, however, results such as the following were obtained:—A 1 per cent. solution of gelatin (1 gm. gelatin in 100 cc. of water) was made by warming at 60° for 10 mins., filtering through cotton wool and then cooling to 15.5°. Measurements within 15 minutes of making the solution gave constant values of  $\rho t$  for pressures varying between 1.2 and 68.8 cm. Hg. After keeping for one hour  $\rho t$  was only constant between about 25–63 cm. Hg pressure, its value rising continuously as the pressure decreased below 25 cm. Hg. After longer intervals of keeping (up to 5 hours) there was less and less constancy of  $\rho t$  at the higher pressures, and the rise of  $\rho t$  with decreasing pressure became more and more marked. Similar results were obtained with a 2 per cent. solution, with which it was further shown that the higher the temperature at which the viscosity is measured the greater is the tendency for  $\rho t$  to remain constant; for example, at 18° there was a sharp rise in  $\rho t$  with decreasing pressure; at 21° the rise was only small, and at 28.5° the value of  $\rho t$  was constant. [Results such as these furnish evidence for the existence of a definite structure in gelatin solutions (compare Weissenberger, *Kolloid Zeitschr.*, 1921, **29**, 113, on "Structures in Disperse Systems."]

Substances which behave similarly to gelatin are—Albumin, insoluble starch, agar-agar, soaps and saponin. Solutions of casein, soluble starch, gum arabic and dextrin, obey Poiseuille's law.

Rothlin concludes his paper with the statement that all viscosity measurements on such substances as gelatin, which have been made with the Ostwald apparatus, are not of very much value. This statement, however, is of too sweeping a character, as will be seen when dealing with von Schroeder's work.

Methods for measuring viscosity which depend on the use of an oscillating disc (Garret, *Phil. Mag.*, 1903, **6**, 374), or on torsion (compare Hatschek, *Kolloid Zeitschr.*, 1913, **13**, 88) have not been used to any great extent in the investigation of gelatin. It is interesting to note that Garret, when using the oscillating disc method, first observed that the introduction of an already solidified portion of the gel to a gelatin solution caused the viscosity to increase more rapidly than before. The falling sphere viscometer (compare Sheppard, *J. Ind. Eng. Chem.*, 1917, **9**, 523; Gibson and Jacobs, *Trans. Chem. Soc.*, 1920, **117**, 473) has not found use in the study of gelatin, although quite recently it has been recommended for this purpose by Fischer (*Zeitschr. angew. Chemie*, 1921, **34**, 153).

The classic work on the viscosity of gelatin solutions is that of von Schroeder (*Zeitschr. physikal. Chemie*, 1903, **45**, 75), as he was the first to show how to obtain concordant results with a substance whose

behaviour depends so much on its previous history and treatment. His results will be referred to a little more fully than will be the case with those of other investigators.

In von Schröeder's experiments a viscometer of the Ostwald type was used, the capillary of which was larger than usual and allowed water to flow out in 40-50 seconds instead of 100 seconds.

Using the finest salt-free gelatin, a weighed quantity was placed in 500 cc. of distilled water and dissolved by warming, never allowing the temperature to rise above 70°. In this way, 1 per cent., 2 per cent. and 3 per cent. solutions were made, taking 10 gms., 20 gms. and 30 gms. respectively for the 500 cc. of water and diluting to 1,000 cc. The solutions were filtered.

In making experiments 200 cc. of a given solution were placed in a small flask fitted with a reflux condenser, and heated in a water bath at 100°. After refluxing for 30 minutes, 5 cc. of the solution were quickly withdrawn in a pipette and placed in a viscometer in a thermostat at 25°; the viscosity was measured 5 minutes after the solution was placed in the viscometer. After another 30 minutes heating at 100° 5 cc. of solution were again withdrawn from the reflux apparatus and treated as before, this process being repeated at definite intervals. Assuming that practically no change took place in the constitution of the gelatin solution within the 5 minutes during which it cooled down from 100° to 25° (other experiments showed that this assumption was justifiable), it was found that the change to a gelatin which no longer sets on cooling,\* and which is spoken of by von Schröeder as hydrolysis, is a regular and irreversible change. The rate at which this hydrolysis takes place was found to be in accordance with the equation:—

$$-dR/dt = K(R - R_0)$$

where  $R$  = viscosity at time  $t$ ;

$R_0$  = lowest possible value of viscosity;

$K$  = constant.

In connection with these particular experiments it should be mentioned that they have been repeated in the British Photographic Research Association's laboratory by Mr. Rawling, B.Sc., under such conditions that the product  $pt$  was constant. The results of von Schröeder were confirmed, thus justifying his use of the Ostwald viscometer under the particular conditions observed.

Shōji (*Biochem. Journ.*, 1919, **13**, 227) repeated von Schröeder's work, using, however, temperatures of 70° and 18° instead of 100° and 25°, and claims that von Schröeder's equation is not valid, but should be replaced by one of higher degree. (Compare also, Loeb, *J. Gen. Physiol.*, 1921, **4**, 97).

Returning to von Schröeder's work:—When the viscosity of each 5 cc. of gelatin solution had been measured after five minutes interval, the solution was maintained in the thermostat at 25° and its viscosity measured at intervals of approximately one hour. It was found that the viscosity of a solution which had been heated to 100° for a short time increased rapidly on keeping; for a solution which had been heated for a longer time at 100° the increase was slower, whilst with solutions which, by prolonged heating at 100°, had attained the lowest viscosity, there was very little or no increase in viscosity and the solution failed to solidify on keeping. These results are indicated by the secondary curves (approximate straight lines) branching off from the main curve in the annexed figure (ordinates = viscosity; abscissae = time in hours. The lowest curve is that for pure gelatin).

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\* This gelatin was first called  $\beta$  gelatin by Traube in 1887, although Wo. Oswald in 1905 (*Pflüger's Archiv.*, 1905, **109**, 277), extended the nomenclature  $\beta$  to any gelatin which had been heated for some time.

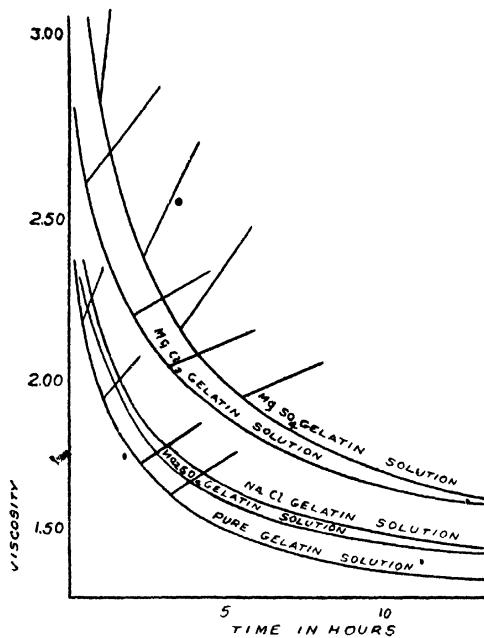


FIG. 8.

In the figure the secondary curves are represented as straight lines, but Shoji (*loc. cit.*) using a 3 per cent. solution, found that the viscosity is not always a linear function of the time. When the sol is heated for a relatively short time (up to 6 hours) the gelation velocity increases with the time, but when it is heated for a relatively longer time the velocity decreases with the time. A 1 per cent. solution always gave gelation velocities decreasing with the time, even with samples of the sol which had only been heated for a few minutes.

From his results von Schroeder concluded that if the ratio  $\Delta R / \Delta t$ , where  $\Delta R$  is the difference in viscosity measured after 5 and 60 minutes, and  $\Delta t$  is the difference in time (=55 mins), is less than 0.0075, the gelatin will not set if it is kept at 25° for 24 hours, whereas if  $\Delta R / \Delta t$  is greater than 0.0095 setting will occur under these conditions.\*

The change in viscosity which takes place when a gelatin solution is kept at a lower temperature after being heated for a short time at 100° is shown by the following figures:—

|                      | After keeping for— |          |          |          |          |
|----------------------|--------------------|----------|----------|----------|----------|
|                      | 5 mins.            | 10 mins. | 15 mins. | 30 mins. | 60 mins. |
| Viscosity at 21° ... | ... ...            | 1.83     | 2.10     | 2.45     | 4.13     |
| " " 24.8° ...        | ... ...            | 1.65     | 1.69     | 1.74     | 1.80     |
| " " 31° ...          | ... ...            | 1.41     | 1.41     | 1.42     | 1.42     |

\* The viscosities given by Schroeder are relatively to those of water (= 1), so that the values of  $\Delta R / \Delta t$  would be different for absolute units.

Thus the secondary changes which take place on keeping at a lower temperature, and which indicate the approach to some kind of equilibrium in the solution, are only marked at the lower temperatures; at 31° the viscosity does not alter over a period of one hour. Similar results were obtained by Levites (*Kolloid Zeitschr.*, 1908, **2**, 208), who points out that for a gelatin solution of given concentration there exists a definite minimum temperature at which the viscosity remains constant. Compare also Bogue (*J. Chem. Met. Eng.*, 1920, **23**, 5).

Loeb (*J. Gen. Physiol.*, 1921, **4**, 97) has investigated the rate of increase of viscosity of gelatin solutions with the time, at different pH's, the temperature being 20°; the solutions were made by heating at 45°. The lower the pH the less the viscosity increases on keeping; for pH = 1·8 and 2·43 the curves showing the relation between the viscosity and the time (up to 60 minutes) are slightly concave to the axis of time; for pH 2·84 and 3·32 the curves appear to be straight lines; for pH 3·80, 4·00, 4·17, 4·55 and 4·74 the curves rise very steeply and are convex to the axis of time.

Davies, Oakes and Brown (*J. Amer. Chem. Soc.*, 1921, **43**, 1526), using a 1 per cent. solution found that the rate of increase of viscosity with ageing of the solution is different at different pH values, the rate of increase being a maximum at pH = 8 - 8·5.

An important contribution to the knowledge of the viscosity of gelatin solutions was made by Arisz in 1915 (*Koll. Chem. Beilhefte*, 1915, **7**, 1-90). Gelatin solutions were prepared by washing commercial gelatin in a stream of water for several days and then drying over concentrated sulphuric acid. The gelatin was then dissolved, at 50°-60°, in glycerol of density 1·176, containing 32 per cent. of water, after which the solutions were filtered. The reason for using glycerol solutions is not given, beyond a reference to an Inaugural Dissertation (*Sol en Geloerstand van Gelatineoplossingen*; Utrecht, 1914). Viscosities were measured in an Ostwald viscometer.

Arisz finds that above 65° there is a continuous decrease in the viscosity of gelatin solutions. At 95° the viscosity of a 10 per cent. sol dropped in 30 minutes to 92 per cent. of the viscosity which it had three minutes after reaching 95°. At 85° the drop was only to 97 per cent. in three minutes, and at 75° to 99 per cent. At 65° there was no change in the viscosity after 24 hours. This change above 65° is irreversible and due to the formation of  $\beta$ -gelatin, that is, to a decomposition of the gelatin.

At temperatures of 65° and below there is no decomposition (over 24 hours) but an equilibrium of some kind is attained in solution. At 65° this equilibrium is reached in less than three minutes and at lower temperatures with ever increasing slowness; below 44° the attainment of the equilibrium is very slow indeed, and the solution slowly becomes a gel. The equilibrium at all temperatures could be approached from both sides by first keeping the solution for some time at a temperature either above or below that being investigated.

Owing to the slow attainment of equilibrium at the lower temperatures, the viscosity of the sol will be less than the equilibrium value if the sol is cooled quickly, say from 65° to 55°, and the viscosity will increase with time. If a sol in equilibrium at say 46° is heated quickly to 55°, the viscosity will be greater than the equilibrium value and will decrease with increasing time, and so on. Until equilibrium is reached, the viscosity of a given gelatin-glycerol sol will thus depend, at temperatures below 65°, on the previous history of the sample and on the time that it has been held at the temperature in question.

This influence of previous history is well illustrated by the following:— A 10 per cent. sol is warmed for a short time at 70° and then cooled to

35° and allowed to gel. If, immediately it gels, the temperature is raised to 44°, it liquefies at once and then slowly gels on keeping. If, however, it is kept at 35° for a long time before being heated to 44°, it does not become liquid at the latter temperature. If a temperature of 20° is used instead of 35°, the gel must be kept for a much longer time at this temperature than at 35° to prevent it becoming a sol when heated to 44°. This is in accordance with the fact that equilibrium will be reached all the more slowly the lower the temperature.

This varying velocity (with the temperature) at which equilibrium is reached is of importance. For example, if a 1·5 per cent. glycerol solution of gelatin is heated at 70° for five minutes and then cooled and kept at 20° it becomes a gel on the third day, whereas if it is kept at 2° it does not gel, even after 14 days. Thus, in contradistinction to ordinary experience, the solution at a higher temperature may be a gel, whilst that at the lower is a sol. If an aqueous sol is preserved for some time at constant temperature (20°) so that equilibrium is attained, and then diluted at the same temperature, the viscosity of the diluted solution gradually decreases, indicating a change in its condition. If the diluted solution is heated to 70° and then cooled to 20°, the viscosity gradually increases on keeping.

Arisz also found that with a 10 per cent. glycerol sol at 50°, repeated passage through the viscometer lowered the viscosity. At 65° there was no difference between successive measurements. Such phenomena have also been observed with aqueous solutions of gelatin (Gokun, *Kolloid Zeitschr.*, 1908, **3**, 84; compare also Bogue, *loc. cit.*) and with solutions of other colloids (Biltz, *Zeitschr. physikal. Chem.*, 1910, **73**, 500; Weissenberger, *Kolloid Zeitschr.*, 1921, **29**, 113), and afford evidence for the existence of an internal structure.

These (aqueous-glycerol) sols showed the Tyndall effect and Arisz finds that the intensity of the scattered light increases with falling temperature and increasing time, provided that the viscosity is below the equilibrium value. For example, when a 1 per cent. sol was cooled from 70° to 20° there was a fourfold increase in the intensity of the scattered light. When a similar sol, previously heated to 70° and then cooled to 18° was kept at the latter temperature, the intensity of the scattered light at the end of nine days was nearly six times the initial value. If, after keeping at 18° for 12 days the sol was warmed to 25° the intensity of the scattered light diminished. Similar results were obtained when the gelatin was present as a gel; also there was no break in the effect when the change from sol to gel took place.

There is therefore parallelism between the change in the Tyndall effect and the changes in viscosity of these sols. These changes afford further evidence of an internal structure existing in the sol.

The increase in the intensity of the Tyndall effect takes place much more rapidly in aqueous than in glycerol solutions, and in agreement with this Van der Heide (*Inaug. Dissert.*, Strassburg, 1887) found that if an aqueous gel is preserved for some time (days) a considerable rise in the liquefying temperature takes place.

#### *Relation between Viscosity and Concentration of Solution.*

Levites (*Kolloid Zeitschr.*, 1908, **2**, 206) first made experiments in this connection, the temperature being 35°, and found that the relation between the viscosity and concentration is linear only in dilute solutions. Loeb (*J. Gen. Physiol.*, 1921, **4**, 73) obtained the following results with isoelectric gelatin:—

[ $\eta/\eta_0$  = ratio of the viscosity of the solution to the viscosity of water.]

| Concentration.<br>% | °     | $\eta/\eta_0$ and $\log.\eta/\eta_0$ * |       |        |       |         | 25°   |             |
|---------------------|-------|--|-------|--------|-------|---------|-------|-------------|
|                     |       | 60°                                    | 45°   | 35°    | 25°   |         |       |             |
| 0·25                | 1·056 | 0·0236                                 | 1·073 | 0·0306 | 1·064 | 0·02697 | 1·090 | 0·0374      |
| 0·5                 | 1·123 | 0·0504                                 | 1·170 | 0·0682 | 1·170 | 0·0682  | 1·200 | 0·0792      |
| 1·0                 | 1·239 | 0·0930                                 | 1·365 | 0·1350 | 1·399 | 0·1457  | 1·474 | 0·1685      |
| 1·5                 | 1·464 | 0·1656                                 | 1·635 | 0·2135 | 1·725 | 0·2367  | 1·890 | 0·2765      |
| 2·0                 | 1·718 | 0·2357                                 | 1·904 | 0·2796 | 2·022 | 0·3057  | 2·345 | 0·3701      |
| 2·5                 | 1·974 | 0·2953                                 | 2·245 | 0·3512 | 2·405 | 0·3811  | 2·945 | 0·4691      |
| 3·0                 | 2·039 | 0·3094                                 | 2·760 | 0·4409 | 3·042 | 0·4832  | 4·019 | 0·6041      |
| 3·5                 | 2·705 | 0·4321                                 | 3·200 | 0·5051 | 3·560 | 0·5514  |       |             |
| 4·0                 | 3·322 | 0·5214                                 | 3·682 | 0·5690 | 4·021 | 0·6043  |       | solidifies. |

\* The figures in italics give the values of  $\log \eta/\eta_0$  and are those given in Loeb's original paper. The figures in ordinary type give the values of  $\eta/\eta_0$  and have been calculated from Loeb's figures. The original paper contains a misprint, the heading of the table being given as  $\eta/\eta_0$  instead of  $\log \eta/\eta_0$ .

The above figures for  $\eta/\eta_0$  give curves which are convex to the axis of concentration, the curvature being all the greater the lower the temperature. For the three higher temperatures the relation between the viscosity and the concentration is given by Arrhenius's formula,  $\log \eta/\eta_0 = k\phi$ , where  $\phi$  is the relative volume of the gelatin in solution and  $k$  is a constant; at 25° the viscosity increases too rapidly for accurate measurement. Einstein's formula,  $\eta = \eta_0 (1 + 2·5 \phi)$ , (*Ann. d. Physik*, 1906, **19**, 289) which gives a linear relation, does not hold (*compare* Hatschek, *Kolloid Zeitschr.*, 1913, **12**, 238; 1920, **27**, 163; Smoluchowski, *ibid.*, 1916, **18**, 190; Arrhenius *Med. K. Vetensk. Nobel Inst.*, 1917, **3**, No. 21). Bogue (*J. Amer. Chem. Soc.*, 1921, **43**, 1764), using solutions varying from 0·01 to 30 per cent. in concentration, also found that Hatschek's formula, which is a modification of Einstein's in which the numerical factor 2·5 is replaced by 4·5 (*Kolloid Zeitschr.*, 1910, **7**, 301; 1912, **11**, 280), does not hold.

On the other hand Davies, Oakes and Brown (*J. Amer. Chem. Soc.*, 1921, **43**, 1526), using the low concentrations of 0·25, 0·5 and 1 per cent. in fairly acid solution (pH 3·6-4·0) state that the viscosity is approximately a linear function of the concentration (*compare* Levites, *loc. cit.*); on the alkaline side of the isolectric point, however, the viscosity increases so rapidly with the concentration that it does not even approximate to a linear function of the latter.

Crupi (*Chem. Met. Eng.*, 1920, **24**, 575) writes discursively on the factors which influence the viscosity of glue, but no definite data are given.

#### *The Effect of Acids and Alkalies on Viscosity.*

Von Schroeder (*loc. cit.*) obtained the following results, which show that the maximum viscosity is attained at low concentrations of both acid and alkali. In view of the work of Loeb and others the maximum is readily explained.

|                                  |      |       |       |       |      |      |      |      |      |
|----------------------------------|------|-------|-------|-------|------|------|------|------|------|
| Normal Conc. of HCl or<br>NaOH.) | 0    | 1/512 | 1/256 | 1/128 | 1/64 | 1/32 | 1/16 | 1/8  | 1/4  |
| Rel. Viscosity (HCl) ...         | 1·40 | 1·55  | 1·76  | 1·68  | 1·58 | 1·42 | 1·25 | 1·17 | 1·12 |
| " " (NaOH) ...                   | 1·40 | 1·52  | 1·60  | 1·79  | 1·62 | 1·38 | 1·25 | 1·10 | 1·10 |

With the exception of a few experiments by Loeb very little work seems to have been done on the influence of ammonia on the viscosity of gelatin. Loeb found that up to a pH of 8·5 the behaviour of ammonium hydroxide was similar to that of sodium and potassium hydroxides. Above pH = 8·5 the action of ammonium hydroxide is abnormal, just as it is with the higher concentrations of acetic acid.

*Action of Salts on the Viscosity of Gelatin Solutions.*

The earlier measurements were made on ordinary commercial gelatins, before the influence of the pH of the gelatin was recognised, and consequently their value is somewhat impaired. Nevertheless the results obtained are far from being of no importance, since it is such gelatin which is used in ordinary commercial processes.

Complications occur, because, as has already been pointed out, the viscosity of the solutions, except under specified conditions, alters with the time, so that a distinction must be made between the initial value of the viscosity as observed immediately after the addition of a salt, and the final value, which is approached only asymptotically. According to the experiments of Levites (*Kolloid Zeitschr.*, 1908, **2**, 208), von Schroeder (*Zeitschr. physikal. Chem.*, 1903, **45**, 75) and Gokun (*Kolloid Zeitschr.*, 1908, **3**, 84), the first of these values follows the general rule of mixtures, that is, salts which raise the viscosity of water affect colloidal solutions similarly, and vice versa.

In connection with this it may be noted that the more the salt slows down the process of gelatinisation, the lower is the temperature at which the viscosity remains constant with the time. For example, Levites found that at temperatures below 26° the viscosity of the gelatin solutions he used increased with the time; in the presence of thiocyanates, however, which retard gelatinisation, the viscosity of the solution remains constant at much lower temperatures than 26°. The converse is also true, that is, if the added substance increases the velocity of gelatinisation the viscosity does not remain constant with time; e.g., in the presence of ammonium sulphate the viscosity is no longer constant even at 30°.

The effect of the addition of various salts on the time-variations in viscosity was studied by Gokun. Only the results for ammonium nitrate are given in detail, and it was found that when small amounts of this salt were added the viscosity of the gelatin solutions increased more quickly than without such, whilst if larger amounts were added (0·32-1·4 N) the viscosity remained almost constant. In very high concentrations (5·6-6·4 N) the viscosity decreased with time, as is the case with suspensoids, probably because at such high concentrations precipitation or coagulation effects play a part. It is doubtful what weight can be attached to these results, since they do not seem to very accurate, and there were considerable temperature variations during the measurements.

Von Schroeder (*loc. cit.*) investigated the influence of the sulphates and chlorides of sodium and magnesium on the rate in change of viscosity of 3 per cent. gelatin solutions when they are heated at 100°, the experiments being carried out similarly to those with pure gelatin. The concentrations of the salts were either 0·5 or 0·25 normal.

As will be seen from the diagram (Fig. 8) all the curves lie higher than those for pure gelatin, that is, the addition of salts raises the viscosity of gelatin, whatever the previous history of the gelatin may be.

All the curves are approximately parallel to the pure gelatin curve, showing that the velocity of the formation of  $\beta$ -gelatin is not appreciably changed by the addition of salts. Moreover, just as with pure gelatin, if  $\Delta R/\Delta t > 0\cdot0095$ , setting occurs at 25° within 24 hours, whereas if  $\Delta R/\Delta t < 0\cdot0075$ , setting does not occur within 24 hours. The value of  $\Delta R/\Delta t$  is increased by sulphates and decreased by chlorides. It should also be noted that the influence of the addition of salt on the ratio  $\Delta R/\Delta t$  bears no relation to the influence of the salt on the viscosity.

The influence of the addition of salt on the rate at which the viscosity of a 1 per cent. solution of gelatin increases at 25°, after a preliminary heating for five minutes at 100°, is illustrated by the following figures, which relate to a solution which had been kept at 25° for one hour after the preliminary heating.

| Concentration.               |      |      |      | Concentration.    |      |      |      |      |
|------------------------------|------|------|------|-------------------|------|------|------|------|
| Salt.                        | N/8  | N/4  | N/2  | Salt.             | N/8  | N/4  | N/2  |      |
| Pure gelatén ...             | 1·78 | 1·73 | 1·78 | Pure gelatin ...  | 1·88 | 1·70 | 1·83 | 1·71 |
| $\text{Na}_2\text{SO}_4$ ... | 2·11 | 2·72 | 9·41 | $\text{NaCl}$ ... | 1·76 | 1·71 | 1·74 | 1·69 |
| $\text{K}_2\text{SO}_4$ ...  | 1·97 | —    | —    | $\text{KCl}$ ...  | 1·80 | 1·67 | 1·60 | 1·51 |
| $\text{Am}_2\text{SO}_4$ ... | 1·95 | 2·21 | 3·32 | $\text{AmCl}$ ... | 1·73 | 1·69 | 1·60 | 1·51 |

| Concentration.               |      |      |      | Concentration. |                     |      |      |      |      |
|------------------------------|------|------|------|----------------|---------------------|------|------|------|------|
| Salt.                        | N/16 | N/8  | N/4  | N/2            | Salt.               | N/8  | N/4  | N/2  |      |
| Pure gelatén ...             | 1·68 | 1·68 | 1·68 | 1·68           | Pure gelatin ...    | 1·65 | 1·68 | 1·76 | 1·76 |
| $\text{LiCl}$ ...            | —    | 1·73 | 1·66 | 1·78           | $\text{NaNO}_3$ ... | 1·63 | 1·57 | 1·56 | —    |
| $\text{MgCl}_2$ ...          | —    | 1·80 | 1·88 | 2·00           | $\text{KNO}_3$ ...  | 1·65 | 1·53 | 1·52 | 1·48 |
| $\text{Li}_2\text{SO}_4$ ... | 1·85 | 1·92 | 2·15 | —              | $\text{AmNO}_3$ ... | 1·61 | 1·52 | 1·49 | 1·45 |
| $\text{MgSO}_4$ ...          | 1·90 | 2·12 | 2·42 | —              |                     |      |      |      |      |

The figures given are viscosity values. The effect of the various salts is best shown by the following table, where the figures represent the difference between the viscosities of the salt-gelatin and pure gelatin.

| —                             | Na        | K     | NH    | Mg    | Li    |       |
|-------------------------------|-----------|-------|-------|-------|-------|-------|
| $\text{N}/16 \text{SO}_4$ ... | ...       | —     | —     | —     | +0·22 | +0·17 |
| $\text{N}/8 \text{SO}_4$ ...  | ... +0·33 | +0·19 | +0·17 | +0·44 | +0·24 |       |
| $\text{N}/4 \text{SO}_4$ ...  | ... +0·99 | —     | +0·48 | +0·74 | +0·47 |       |
| $\text{N}/2 \text{SO}_4$ ...  | ... +7·63 | —     | +1·54 | —     | —     |       |
| $\text{N}/8 \text{Cl}$ ...    | ... -0·12 | -0·08 | -0·15 | +0·12 | +0·05 |       |
| $\text{N}/4 \text{Cl}$ ...    | ... +0·01 | -0·03 | -0·01 | +0·20 | -0·02 |       |
| $\text{N}/2 \text{Cl}$ ...    | ... -0·09 | -0·23 | -0·23 | +0·32 | +0·10 |       |
| $\text{N} \text{Cl}$ ...      | ... -0·12 | -0·20 | -0·20 | —     | —     |       |
| $\text{N}/8 \text{NO}_3$ ...  | ... -0·02 | -0·00 | -0·04 | —     | —     |       |
| $\text{N}/4 \text{NO}_3$ ...  | ... -0·11 | -0·15 | -0·16 | —     | —     |       |
| $\text{N}/2 \text{NO}_3$ ...  | ... -0·20 | -0·24 | -0·27 | —     | —     |       |
| $\text{N} \text{NO}_3$ ...    | ... -0·22 | -0·25 | -0·25 | —     | —     |       |

The results are shown in the following diagram.—

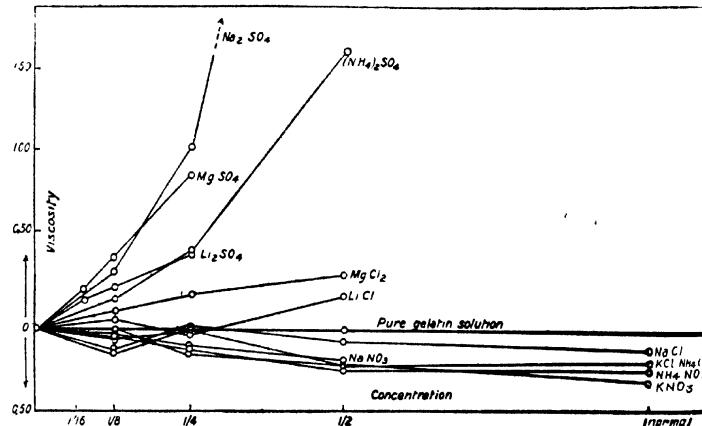
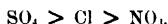


FIG. 9.

The results show that sulphates in all concentrations increase the viscosity of gelatin, whilst chlorides and nitrates decrease it, with the exception of magnesium and lithium chlorides in higher concentrations. The exact concentration of the salt, however, plays an important part, especially with the chlorides, which in medium concentration (about N/4) show a maximum viscosity which sometimes exceeds that of pure gelatin. Generally speaking, the influence of the added salt on the viscosity of gelatin is the same as its influence on the viscosity of water (Wagner, *Zeitschr. physikal. Chem.*, 1890, 5, 31).

If the anions of the added salts are arranged according to their effect the order is:—



In the case of the cations, variations occur with different concentrations. If the values are taken for N/8 solutions, the sulphates and chlorides arrange themselves as follows:—



Statements such as the above indicate the difficulties which occur in connection with the Hofmeister series when no account is taken of the pH of the solutions.

In one of a series of papers dealing chiefly with albumin, Pauli and Falek (*Biochem. Zeitschr.*, 1912, 47, 270) give some results obtained with a 0·3 per cent. solution of gelatin, the gelatin having previously been carefully freed from electrolytes. The temperature of experiment was 30°, since at room temperature, the gelatin owing to its purity flocculated out. To gelatin solutions which were either acid or alkaline were added various sodium salts so that the concentrations of the latter were 0·01N and the viscosities measured.

| Salt            | ·005N HCl | ·01N HCl | ·01N NaOH |
|-----------------|-----------|----------|-----------|
| —               | —         | —        | —         |
| Chloride ...    | 1·39      | 1·32     | 1·30      |
| Bromide ...     | 1·23      | 1·25     | 1·22      |
| Nitrate ...     | 1·23      | 1·21     | 1·22      |
| Iodide ...      | 1·22      | 1·22     | 1·22      |
| Thiocyanate ... | 1·21      | 1·21     | 1·21      |
| Sulphate ...    | 1·14      | 1·16     | 1·20      |

[In the original paper the results are given to more than two places of decimals.]

An interpretation of these results, based on Loeb's work, can easily be given. The acid solutions will have a pH less than 4·7 and the gelatin will be present as cation. The salts of the monobasic acids, in equivalent concentration should therefore have the same effect on viscosity, decreasing it to the same extent because of the suppression of dissociation. The figures show that this is the case.

The sulphate should have a much greater effect and again the experimental result is in agreement.

With the alkaline gelatin, since the gelatin is present as anion, gelatinates being formed, all the salts, being present in equivalent concentration, should have the same effect: the experimental figures show that this is the case.

#### Action of Non-Electrolytes.

Very little has been published in the literature with respect to the action of non-electrolytes (alcohol, sugar, etc.), on the viscosity of gelatin, although their action on other emulsoids (albumin, etc.) has been

extensively studied. In low concentrations non-electrolytes usually change the viscosity of emulsoids only to the extent in which they increase the viscosity of the pure dispersion medium. (Levites, *Kolloid Zeitschr.* 1907, **2**, 210; Pauli and others).

Bogue (*J. Chem. Eng.*, 1920, **23**, 5) found that methyl, ethyl and amyl alcohols increase the viscosity of gelatin, but his method of experiment was such that the results can only be considered to be of a qualitative nature.

(Note.—The effect of substances which harden gelatin is not dealt with in this section.)

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### SWELLING

Except in a few experiments by Hurter, which are detailed in a paper read by Hurter and Driffield before the Royal Photographic Society in 1898, strips or discs of gelatin, or else powdered gelatin, have been used in the investigation of swelling.

The method of experiment usually employed is based on that first used by Hofmeister (*Archiv. Exper. Path. und Pharm.*, 1890, **27**, 395; 1891, **28**, 210). As improved by Wo. Ostwald (*Pflüger's Archiv.*, 1905, **108**, 563, 1905, **109**, 277; 1906, **111**, 581; *Kleines Praktikum der Kolloidchemie*, 2nd Edition, p. 82) the method consists essentially of casting, from a warm solution, a layer of gelatin of uniform thickness. From this layer, strips or discs of definite size are cut and then carefully dried to constant weight, in such a way that crinkling does not take place. For measuring the swelling the weighed strips or discs are put into a dish containing water or the solution under investigation, after a given time they are taken out, carefully dried with soft filter paper, or better with a thin porous cloth which has become very soft by repeated washing, and then weighed again.

Miss Schreve (*J. Franklin Inst.*, 1919, **187**, 139) has modified the method by casting the gelatin in small cylindrical glass dishes with vertical sides and accurately made flat bottoms, and supplied with ground cover glasses to fit. During all the operations (swelling, and drying with filter paper) the gelatin remained in the same dish. When discs of gelatin were used, as in Wo. Ostwald's method, they were always handled with gold tipped forceps and were weighed in weighing bottles.

In still another modification of the method, Miss Lloyd (*J. Soc. Leather Trades' Chemists*, 1920, **4**, 164) used discs of Coignet's "Gold Label" gelatin, of known water content. These were placed in Gooch crucibles where they remained at all stages of the experiment. Experimental liquids were contained in glass bottles with wide necks and ground-in stoppers, the Gooch crucibles being suspended in these by a harness of fine thread. Both crucible and gel were dried rapidly with filter paper before each weighing.

From the above it will be seen that in some cases the dried gelatin, and in other cases the concentrated gel, is used to determine the swelling. The latter method is advocated by Chiari (*Biochem. Zeitschr.*, 1911, **33**, 167) as it is believed that in this way the effect of the elastic forces, etc., which may be present, is reduced to a minimum. No matter what method is used, the weight of water taken up in swelling is calculated on the dry weight of gelatin, that is, on the weight of the anhydrous gelatin.

With powdered gelatin the volume method of measuring swelling may be used. The powder is allowed to swell in a tube of known and constant diameter, the extent to which it rises in the tube then being a measure of the amount of swelling.

#### *Swelling of Gelatin in Water.*

A large amount of work on the swelling of gelatin was done before the necessity of first purifying the gelatin was realised. One of the first to point out this necessity was Chiari (*Biochem. Zeitschr.*, 1911, **33**, 167) who showed that gelatin which had been dialysed against running water for several weeks and was then brought into equilibrium with ordinary distilled water, underwent a decided de-swelling when put into conductivity water, that is, into pure water free from carbon dioxide. Chiari's statement that the maximum swelling of pure gelatin can only be found in the purest conductivity water must be modified in view of the relation which is now known to hold between the pH and the swelling.

Before the complexity of the phenomena associated with the swelling of gelatin was realised, attempts were made to express the results by

mathematical formulæ. Hofmeister (*loc. cit.*) put forward a formula from which it would follow (Pauli, *Pflüger's Archiv*, 1897, **67**, 219) that the velocity of swelling is given by the equation

$$\frac{dW}{dt} = K (P - W)^2$$

where  $W$  = weight of water absorbed by unit weight of gelatin in time  $t$ .

$P$  = maximum weight of water which unit weight of gelatin will absorb,

$K$  = constant.

The velocity of swelling should therefore be proportional to the square of the swelling deficit. Such a result does not seem very probable, and Pauli put forward a simple equation  $dW/dt = K'(P-W)$  which was found to agree with Hofmeister's results within the degree of accuracy (5 per cent.) claimed by the latter. Both formulæ lead to the conclusion that the velocity of swelling is at a maximum at the instant of immersion and therefore decreases with time, this corresponding with experimental facts. Compare also, Sheppard and Mees, *Investigations on the Theory of the Photographic Process*, 1907, p. 85.

As was pointed out in the section on viscosity, Wo. Ostwald (*Pflüger's Archiv*, 1905, **109**, 277) extended the meaning of  $\beta$ -gelatin to comprise any gelatin which has a previous thermal history, that is, which has previously been heated to about  $100^\circ$  for a time. He found that both the rate and intensity of swelling are greater for  $\beta$ -gelatin than for ordinary gelatin.

The complexity of the phenomenon of the swelling of gelatin in water is illustrated by the following quotation from Bancroft's book on "Applied Colloid Chemistry" and from Arisz's paper on swelling (*Koll. Chem. Beihefte*, 1915, **7**, 47). Arisz's paper is full of details which it is almost impossible to summarise, and which can only be fully appreciated by reference to the original.

"If dried gelatin is placed in cold water it swells considerably and may take up 10 times its weight of water, but there are no experiments to show that it would ever go up, say to an 8 per cent. gel. On the other hand it is possible to start with an 8 per cent. gel and dry it to a 96 per cent. gel, after which it will take up water rapidly to an 8 per cent. gel. This means that the structure of the gelatin plays an important part in the rate of swelling. This is confirmed by some unpublished preliminary results by Mr. Cartledge. Gelatin gels were made up containing 8, 16, 24 and 32 per cent. of gelatin. These were all dried at room temperature to about 96 per cent. concentration. When water was added, each swelled rapidly to the original concentration and then took up water slowly. If these results are accurate, it means that the four 96 per cent. gels were all different and that the 8 per cent. gel did not become like the 16, 24 or 32 per cent. gel while being dried."

Results of an analogous nature were obtained by Arisz. An 80 per cent. gel was liquefied at  $70^\circ$ , portions diluted to various concentrations and then each cooled again to a gel. The blocks used for measuring the swelling were  $33 \times 7 \times 4$  mm., and the experiments were carried out at  $20^\circ$ . It was found that a 0.5 per cent. gel disintegrates completely in water, a 10 per cent. gel goes to a 2 per cent. gel in 4 days, a 20 per cent. gel to a 6 per cent. one, a 50 per cent. gel to a 16 per cent. one, and an 80 per cent. gel to a 20 per cent. one. At the end of 4 days, the rate of swelling of the 80 per cent. gel, which is now a 20 per cent. one, is so slow that the swelling curve is approximately parallel to the time axis, whereas the original 20 per cent. gel swelled very rapidly when immersed in water. It is thus possible to have two gels of the same concentration, one of which will hardly swell, whilst the other swells rapidly.

The effect of the previous history of the gel on the swelling is thus very marked. Arisz does not mention how long or at what temperature, the gels were kept before using, but his previous experiments on the Tyndall effect showed that the solid gels underwent a change in structure on keeping, and this change is undoubtedly connected with the different degrees of swelling. In later experiments Arisz shows that in order to obtain comparative results it is absolutely necessary to use gels having the same history, freshly prepared gels swelling more rapidly and to a greater extent than old ones. The greatest diminution in the swelling power on keeping occurs when the gel is kept at the same temperature as that at which its swelling is measured.

Further evidence of change in structure is given by Arisz's experiments on intermittent soaking. If two identical gels are placed in water and one is kept in the water 6 days, while the other is only in the water for the first two and last two days, the temperature being kept constant all the time, the amount of swelling will be the same within the limits of experimental error. While the partially swollen gel is out of the water, some change in structure must take place such that it takes up water when re-immersed, so much more rapidly than the other that the total swelling is the same in the two cases, although one gel was soaked twice as long as the other.

Arisz concludes from his experiments that swelling takes place in two stages. During the first stage there is a period of rapid swelling, at the end of which maximum swelling has practically been attained. The second stage represents a series of swelling equilibria which alter slightly with the changes which take place in the internal condition of the gel. It is doubtful whether much stress can be laid on these two stages, since their observance, or otherwise, depends on the thickness of the plates of gelatin used. In fact, one of the difficulties of swelling experiments is the influence of the thickness of the gelatin plate or disc used. Holmester recognised this, and obtained concordant results only with thin plates. Moreover, a swelling maximum is often referred to in the literature, but the existence of such a maximum has never been proved. In connection with this question reference may be made to some experiments by Brotman (*J. Soc. Leather Trades' Chemists*, 1921, **5**, 226) in which he shows that the imbibition of water by a sample of gelatin can be increased by dispersion of the gelatin. Pure air-dry gelatin swells to "maximum" amount in about 24 hours, the maximum varying according to the variety of gelatin used. If the equilibrium gel is then dispersed by placing the test tubes containing it in hot water (at about 80°) for two minutes, and then allowed to set again, further swelling will take place on immersion in the previous swelling water. The gel structure thus plays an important part in determining the capacity for imbibition of water.

Two other results of Arisz are as follows:—

(1) A gel which is in swelling equilibrium at 10°, de-swells when it is warmed to 20°. If it is in equilibrium at 20° and is then cooled to 10° it takes up water more rapidly than before.

(2) If a gel, whose previous history has been so chosen that it takes up water more quickly at 10° than at 20°, is preserved out of water for some time, it undergoes such changes that its behaviour on swelling is reversed.

Katz (*Koll. Chem. Berichte*, 1917, **9**, 1482) has investigated the quantitative laws of swelling of a large number of different colloids and proteins, among which was gelatin. In order to avoid the influence of previous history, only samples which had undergone considerable ageing were used.

If the degree of swelling ( $i$ ) is defined as the water content in grains per gram of dry substance, that is,  $i = w/g$ , where  $w$  = weight of water,

and  $q$  = weight of gelatin, and  $h$  is taken as the ratio of the vapour pressure of the gel to that of pure water at the same temperature, the relation between  $i$  and  $h$  is given by an S-shaped curve, as shown in the diagram.

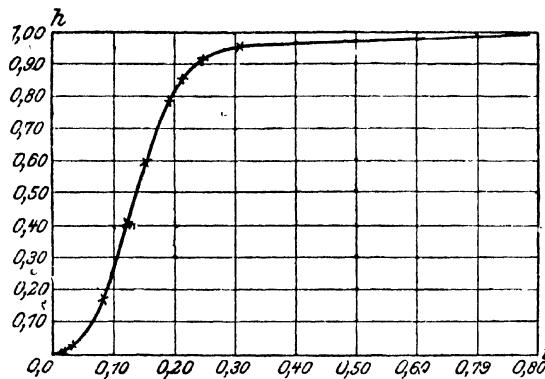


FIG. 10.

The curve becomes asymptotic to the line  $h = 1$ . The almost horizontal shape of the last portion of the curve shows that a very small lowering of the vapour pressure will correspond with a very large alteration in the degree of swelling.

If the heat of swelling,  $W$ , is defined as the quantity of heat (in calories) which is evolved when one gram of gelatin takes up  $i$  grams of water, the relation between  $W$  and  $i$  is given by the equation

$$W = Ai / (B + i)$$

where  $A$  and  $B$  are constants. This implies that at the initial moment of the swelling process, the heat liberated by one gram of gelatin per gram of water absorbed will be equal to the ratio  $A : B$ . This ratio is found to be very large and equal to 230 cals.\* The evolution of heat is thus practically limited to the initial stages of swelling, a conclusion which was also drawn by Rosenbohm, who used an ice calorimeter in his experiments. This explains an apparent contradiction to Wiedemann and Lüdeking's result (*Wied. Annalen*, 1885, 25, 145) that the average heat of swelling at 18·4° is +5·7 cals. per gram.

Before leaving the question of the swelling of pure gelatin, reference must be made to what is known as the von Schroeder problem. A disc or plate of gelatin which has absorbed as much water as possible when immersed in water, loses weight when placed in a space saturated with water vapour at the same temperature. On the other hand a dry plate of gelatin swells up when exposed to saturated water vapour; when it has reached equilibrium in the water vapour, however, it will, if placed in water, exhibit further swelling. Again, if a strip of gelatin is placed vertically with the lower part in the water and the upper part in vapour, the lower will swell much more than the upper part. This is held to be a contradiction of the second law of Thermodynamics. It is doubtful, however, whether the phenomenon is a real one. As far back as 1791, Deluc (*Phil. Trans.*, 1791, 81, 1, 389) showed that the air above a water surface is only really saturated with water vapour to a height of 2·55 cm., so that equilibrium would not be obtained under the conditions observed by

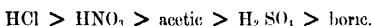
\* Arisz himself did not investigate the heat of swelling of gelatin, but used the results of Rosenbohm *Koll. Chem. Beihefte*, 1914, 6, 177) which agree with the formula given.

von Schroeder. Later experiments of Wolff and Büchner (*Proc. K. Akad. Wetensch. Amsterdam*, 1913, **15**, 1078; 1914, **17**, 92) indicate that the von Schroeder phenomenon does not exist under conditions of true equilibrium. (See also, Bancroft's *Applied Colloid Chemistry*, p. 75, seq.) The results obtained by Arisz, that a very small lowering of the vapour pressure corresponds with a very large alteration in the degree of swelling will also explain why a real equilibrium was not obtained in von Schroeder's experiments. Compare also: Shull and Shull, *Am. J. Botany*, 1920, **3**, 318.

#### *Influence of Acids and Alkalies on the Swelling of Gelatin.*

Hofmeister (*Archiv exper. Path. und Pharm.*, 1890, **27**, 395) and Spiro (*Hofmeister's Beiträge*, 1904, **5**, 276) early investigated the influence of acids and alkalies on the swelling of gelatin, but their methods of experiment were improved and their results extended by Wo. Ostwald in 1905 (*Pflüger's Archiv*, 1905, **108**, 563). Ostwald showed that the swelling depends in a complex manner on the concentration. With very weak concentrations (N/210 HCl, N/100 KOH) there is a minimum swelling as compared with water, the swelling then increasing to a maximum at N/40 HCl or N/36 KOH. The minimum with very weak acid is understandable, since the gelatin used would probably be on the alkaline side of the isoelectric point, but that with N/100 KOH, assuming the same gelatin was used, does not agree with the results of Loeb, Pauli and others. Ostwald's opinion in 1905 seemed to be that the minimum in the swelling curve corresponded with the maximum in the viscosity curve given by von Schroeder, whereas according to recent ideas, "minimum and maximum should correspond with minimum and maximum in the two curves." This correspondence has recently been emphasized by Lüters and Schneider (*Kolloid Zeitschr.*, 1921, **28**, 1) in their experiments on the viscosity and swelling of gluten and gliadin in the presence of lactic acid.

The influence of equivalent solutions (0·1N) of various acids on the swelling is indicated by the following series —



With alkaline hydroxides the order is



The different results which are obtained when the swelling under the influence of various acids is compared with respect to (a) the equivalent concentrations of the acid, (b) the hydron concentrations in the acid solutions, is well illustrated by the work of Chiari (*Biochem. Zeitschr.*, 1911, **33**, 167), who used solutions of hydrochloric, acetic and lactic acids in which the normalities varied from  $5 \times 10^{-4}$  to  $1 \times 10^{-5}$ , whilst the hydron concentration varied from  $10 \times 10^{-5}$  to  $1 \times 10^{-5}$ . If the curve of swelling is plotted against the concentration of the acid, hydrochloric acid seems to have a much greater effect than lactic acid, and lactic acid greater than acetic acid; if plotted against the hydron concentration, then acetic acid is greater than lactic acid, which is greater than hydrochloric acid. Results such as these show the necessity of determining the pH of the gelatin-acid solutions itself, and not simply of the acid solution before, the gelatin was added.

In the paper just mentioned Chiari is the first to suggest that the position of minimum swelling of gelatin corresponds with the gelatin being at the isoelectric point.

Ehrenberg (*Biochem. Zeitschr.*, 1913, **53**, 356) gives a long account of numerous experiments, the results of which he is unable to explain, but which can readily be explained in the light of later work.

Fischer and Hooker (*J. Amer. Chem. Soc.*, 1918, **40**, 272) have investigated the swelling of gelatin in solutions varying in composition from ortho-phosphoric acid to sodium hydroxide, through the intermediate

mixtures of sodium dihydrogen and disodium hydrogen phosphate, similarly with citric acid in place of phosphoric acid. They find that minimum swelling occurs in (a) a solution having the composition of sodium dihydrogen phosphate, (b) a solution consisting of a mixture of 40 per cent. mono-sodium citrate and 60 per cent. disodium citrate. Atkin (*J. Soc. Leather Trades' Chemists*, 1920, **4**, 248) in discussing these results shows that the acidity of these solutions corresponds approximately with the isoelectric point of gelatin, where the swelling should be at a minimum.

The most recent English work on the swelling of gelatin is that of Miss Lloyd (*Biochem. J.*, 1920, **14**, 147; *J. Soc. Leather Trades' Chemists*, 1920, **4**, 163). Solutions in boiled distilled water of the gelatin used gave a pH = 5.25 at concentrations of 0.2 and 0.5 per cent., so that the gelatin contained a small quantity of base. The ash content was 1 per cent. and consisted mainly of calcium sulphate with traces of phosphates and chlorides.

In three experiments where the ratios, "dry weight of gelatin, Volume of 0.001N HCl" were 0.8/100, 0.8/1000 and 0.8/10000 respectively, the swelling was all the greater as this ratio decreased, in the first two, cases the swelling attaining a maximum value,\* whilst in the third the gelatin went into solution. The swelling thus seems to depend on the ratio of gelatin to acid, and this only can be explained by the assumption that different amounts of acid are absorbed by the gelatin in the three different experiments, the pH of the gelatin, and consequently the swelling thus being different in the three cases. That this is so is evidenced by the fact that the pH of the external solution changes from the original value of 3.0 to 4.4, 3.57 and 3.25 in the respective experiments. The actual pH of the gelatin itself was not measured by Miss Lloyd, but it is known that the swelling curve falls sharply away from the maximum point at pH = 2.4 (Atkin, *loc. cit.*), so that small alterations in the pH of the gelatin will cause large alterations in the amount of swelling.

In other experiments the swelling of gelatin in different concentrations of hydrochloric acid and sodium hydroxide was determined. The results are best indicated by the annexed diagram (Fig. 11), which represents the swelling at different pH values after an arbitrary period of 48 hours. Unfortunately in these experiments, also, the ratio of dry gelatin was not kept constant, but varied from 1/2200 to 1/3750, so that the curve cannot be taken as quantitatively accurate.

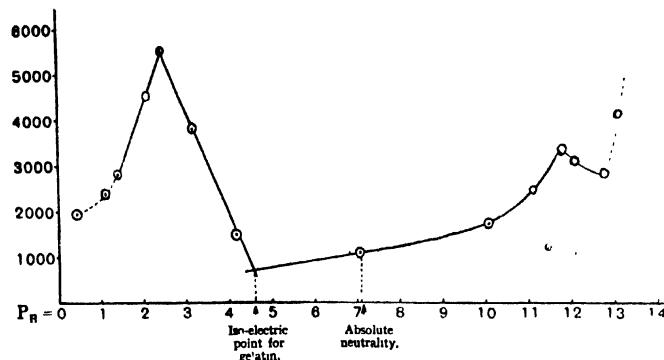


FIG. 11.

\* Miss Lloyd states in this paper that "even after 2 or 3 months the swollen gels still show a steady increase in weight," that is, a true maximum of swelling is not obtained.

If solutions of gelatin in hydrochloric acid or in sodium hydroxide which have been made by allowing the gelatin to stand in contact with these liquids in the cold are examined at the moment of solution, there is no chemical evidence that there has been any breakdown of the protein molecule. From the hydrochloric acid solution, the gelatin can be recovered with its original properties, so that the reaction:—  
 gelatin + HCl  $\rightleftharpoons$  gelatin hydrochloride, is reversible. From the sodium hydroxide solution, however, the gelatin is not recovered with its original properties, so that the formation of sodium gelatinate is not reversible.

In some further interesting experiments Miss Lloyd deals with the behaviour of gels, swollen in acid or alkaline solution, when they are transferred to a saturated water vapour phase. The results have bearing chiefly on the structure of gelatin gels.

C. R. Smith (*J. Amer. Chem. Soc.*, 1921, **43**, 1350) has investigated the swelling of gelatin (and the osmotic pressure of gelatin solutions) in the presence of acids and alkalis and obtained results which are in accordance with the later ones of Loeb, although he criticises adversely Loeb's earlier work. Kuhn (*Koll. Beiträge*, 1921, **14**, 147) has studied the swelling of gelatin in aqueous solutions of some 44 organic acids and phenols. Great care was taken to purify the acids and phenols used, but the gelatin was an ordinary commercial powdered sample, containing 3.11 per cent. of ash. He finds that the swelling curves are in accordance with the ordinary adsorption isotherm

$$x = qc^n$$

Where  $x$  = quantity of water absorbed  
 $c$  = concentration of the acid.  
 $q$  and  $n$  = constants.

No relation between  $q$ ,  $n$  and the dissociation constant of the acid was found to exist. It is remarkable that in a paper of such recent date no account is taken of the pH of the solutions, nor is reference made to Loeb's work.

Reference may be made to the following papers which deal with the mechanism of swelling in acid and alkaline solutions, more especially from the theoretical point of view; the substance used was blood fibrin, and not gelatin. Tolman and Stearn (*J. Amer. Chem. Soc.*, 1918, **40**, 264), Tolman and Bracewell (*ibid.* 1919, **41**, 1503), Bracewell (*ibid.* 1919, **41**, 1511).

#### *Influence of Salts on the Swelling of Gelatin.*

Hofmeister (*loc. cit.*) showed that with sodium salts the swelling decreases in the order:—Bromides > nitrates > chlorates > chlorides > acetates > tartrates > citrates > sulphates. To these may be added:—thiocyanates > iodides > bromides, etc.

For such experiments it is of importance to use a gelatin which is as neutral as possible, and it is moreover of importance to note that the concentration of the salt solutions employed has an influence on the result. With 0.125-molar solutions Wo. Ostwald states that sulphates, acetates, tartrates and oxalates lower the swelling maximum, whereas the maximum is raised by nitrates, chlorides, bromides, iodides, etc.

The influence of different concentrations of the same salt is a very complicated one. Wo. Ostwald (*Pflüger's Archiv*, 1906, **111**, 581), using sodium chloride, found that with M/8 solutions there was a maximum swelling, which was followed by a minimum swelling with M/4 solutions. These maximum and minimum points are claimed to be the mirror images of the minimum and maximum points observed by von Schroeder in the viscosity curves. Similar results were obtained with the chlorides of potassium, ammonium, lithium, calcium, barium and magnesium, and with the nitrates of potassium, sodium and ammonium, and the state is made that there is a complete and perfect parallelism or mirror

image effect between the swelling and viscosity. Ostwald, however, seems to have strained the interpretation of the results to a considerable extent in order to make them fit in with the mirror image theory; swelling and viscosity results should run parallel with each other. The fact that calcium chloride causes much greater swelling than sodium chloride indicates that Ostwald must have used an acid gelatin, so that his results are not necessarily comparable with those of von Schroeder. Thus again is the necessity emphasised of knowing the pH of the gelatin in all experiments carried out.

A further complication which may occur in all experiments with neutral salts is that owing to adsorption effects by the gelatin, hydrolysis of the salt may occur (unequal adsorption of the ions); owing to this hydrolysis the gelatin becomes acid or alkaline, as the case may be, and the swelling is thereby affected. As a matter of fact Bancroft (*Applied Colloid Chemistry*) criticises Loeb's earlier work from this point of view, but the mass of data now collected by Loeb in support of his ideas is such that it is doubtful whether Bancroft's criticisms are valid.

A factor which is of importance and of which sufficient account has not been taken in swelling experiments is as follows:—It is possible that if the salts are dissolved in the gelatin itself before it is swollen, the results may be quite different from those which would be obtained if the pure gelatin were used. Ehrenberg (*Biochem. Zeitschr.*, 1913, **53**, 356) states that the action on the swelling, of salts, acids and bases dissolved in gelatin itself, is the same as if they were in the outer solution, whereas Miss Schreve (*J. Franklin Inst.*, 1919, **187**, 319) is of opposite opinion. She states that when dry gelatin is made up to a gel with any of the solutions given below, instead of with water, or when a gelatin gel containing 72 per cent. of water is allowed completely to absorb 1/5-3/10 of its own volume of these solutions at 10°-30°, the imbibitional properties of the gelatin are so changed that the rate of swelling is increased. These solutions are:—M-NH<sub>4</sub>Cl, M-NaCl, M-NaBr M- and 2M-C<sub>2</sub>H<sub>5</sub>OH, M- and 2M-Na citrate, M- and 2M-Na tartrate, M- and M/2-Na<sub>2</sub>SO<sub>4</sub>. When, however, a dry gelatin is made up to a gel with water and placed in these solutions and the rate of swelling compared with the rate of distilled water, the results are found to agree with those of Hofmeister, that is the rate of swelling is greater for NH<sub>4</sub>Cl, NaCl and NaBr than for distilled water, and less for C<sub>2</sub>H<sub>5</sub>OH, Na citrate, Na tartrate and Na<sub>2</sub>SO<sub>4</sub> than for distilled water. "It is therefore not safe to draw conclusions regarding the effect which substances within a gel exert on its swelling capacity, when the evidence for such a conclusion lies in the known effect of an external solution on the same substance or other gels."

Ehrenberg (*loc. cit.*) carried out a large number of swelling experiments with different salts, and with different combinations of salts, but it is difficult to know what value to assign to his results, since no methods are given for the preparation of the gelatin gels. Results for which he can find no explanation are readily explicable in the light of later work.

Lenk has published two long papers on (a) the action of single electrolytes on swelling (*Biochem. Zeitschr.*, 1916, **73**, 15), and (b) the action of combinations of electrolytes on swelling (*ibid.*, 1916, **73**, 58), but since he used gelatin discs which had already imbibed water to 80-85 per cent., and his method of experiment was very crude (*compare* W. Ostwald, *ibid.*, 1916, **77**, 329), the results obtained are of very little value. It is pointed out, however, that the order of the Hofmeister series depends on the concentrations of the solutions used.

Mutscheller (*J. Amer. Chem. Soc.*, 1920, **42**, 2142) finds that gelatin shows a maximum swelling (and viscosity) in the presence of 0.28-M zinc sulphate. Since zinc sulphate is hydrolysed in solution, this effect may be connected with the acidity of the solutions, but no measurements of the pH are given.

In a previous section, when dealing with Loeb's work, it was shown that the addition of a salt to a gelatin on the acid side of the isoelectric point reduces the viscosity of the gelatin solution; the same is true of the swelling of gelatin. Procter made use of this effect in determining the amount of acid retained by gelatin swollen in hydrochloric acid (compare also, Smith, *J. Amer. Chem. Soc.*, 1921, **43**, 1350). Bechold (*Colloids in Pathology and Medicine*, 1919, p. 69) cites an experiment in which 0·78 gram of gelatin swelled in 100 cc. of 0·05N-HCl until it weighed 14·61 grams. The weight was only about 7 grams when the solution was also half molecular with respect to potassium chloride, and 2·84 grams when half molecular with respect to potassium citrate.

*Influence of Organic Substances on the Swelling of Gelatin.*

Fischer and Sykes (*Kolloid Zeitschr.*, 1914, **14**, 215) found that non-electrolytes (sucrose, lactulose, dextrose, methyl and propyl alcohols, propylene glycol and acetone) reduce the swelling of gelatin, the effect increasing with increasing concentration of non-electrolytes. This is in contradistinction to the action of electrolytes, which with increasing concentrations have less and less effect. Carbamide (urea), which acts as an organic base, increases the swelling (Fischer and Sykes, *Kolloid Zeitschr.*, 1915, **16**, 129). The addition of hydrochloric acid, sodium chloride or magnesium sulphate increases the swelling due to carbamide. Pyridine also increases the swelling, owing to the alkalinity of the solution; the addition of hydrochloric acid to the pyridine solutions first decreases the swelling (removal of OH<sup>-</sup> ions) and then increases it (addition of H<sup>+</sup> ions). Sodium chloride and dextrose reduce the swelling due to pyridine.

FIRST REPORT  
OF THE  
ADHESIVES RESEARCH COMMITTEE

TO THE ADVISORY COUNCIL TO THE COMMITTEE OF THE PRIVY  
COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH

GENTLEMEN,

THE Adhesives Research Committee appointed by you in December, 1919, to continue the work of the Adhesives Committee of the Conjoint Board of Scientific Societies, beg to submit herewith their First Report.

The Committee have found it desirable to include in this Report an account not only of the investigations conducted under their direction, but also of the unpublished work of the original Committee of the Conjoint Board. The Report is thus a comprehensive summary of the work from its inception in 1918 up to the end of 1921.

Appendix I to the Report is a descriptive bibliography of gelatin which has been prepared and revised by Dr. T. Slater Price, O.B.E., Director of Research of the British Photographic Research Association. Your Committee believe that this bibliography will be of great value to workers in the subject. The second Appendix has been prepared for the purpose of indicating the bearing, upon the structure of gelatin gels, of the recent work of Professor McBain and his collaborators on the nature of soap solutions and gels.

MERVYN O'GORMAN,  
*Chairman.*

## I.—INTRODUCTION

1. Towards the end of the war a shortage of glues and cements was threatened; increasing demands for these products coincided with diminishing supplies of the raw materials. The demand was chiefly due to the number of aircraft under construction, while the necessity for conserving food supplies limited the amount of casein available for the production of cements. The position was such that, early in 1918, it was estimated that within a year the shortage of glue would seriously limit the output of aircraft. In the latter part of 1917, however, the probability of a shortage had already been foreseen and the attention of the Engineering War Committee of the Royal Society was called by a memorandum\* from one of its members† to the necessity for the scientific study of adhesives. This Committee referred the matter to the Conjoint Board of Scientific Societies which body called together an Adhesives Committee representative of the numerous aspects of the subject, both theoretical and practical.

2. In March, 1918, a deputation from the Adhesives Committee explained to Sir William Weir the nature of the scientific assistance which, it was felt, the Committee could give to the Air Ministry and eventually, upon the recommendation of the Air Group of the Ministry of Munitions, the latter Ministry accepted the co-operation of the Committee. The Ministry of Munitions subsequently assigned to the Committee a sum not to exceed £1,000, to cover the working expenses for one year. The Ministry also nominated representatives to serve on the Committee and agreed to place at its disposal all the available technical information on the subject.

3. The constitution of the Adhesives Committee at this time was as follows :—

Lieut.-Colonel M. O'Gorman, C.B. (*Chairman*)  
Sir Dugald Clerk, K.B.E., F.R.S.  
Professor W. E. Dalby, F.R.S.  
Professor J. B. Farmer, F.R.S.  
Professor P. Groom (Tech. Dept. Air Group)  
Mr. J. S. Highfield.  
Sir Herbert Jackson, K.B.E., F.R.S.  
Captain Kaye (Aircraft Production Dept.)  
Lieut. J. C. Kernot, D.Chem.  
Professor W. H. Lang, F.R.S.  
Major A. Robertson, R.A.F.  
Sir J. J. Thomson, O.M., F.R.S. (*Chairman, Conjoint Board*).  
Mr. C. J. Wilson.  
Captain G. J. Woods (Supply Dept., Air Group).  
Professor W. W. Watts, F.R.S. (*Secretary*).

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\* Subsequently published in "Aeronautics," January 2nd, 1918.

† Lieut.-Colonel M. O'Gorman, C.B.

Having in view the conserving of the existing supplies of raw materials for adhesives and cements, the Committee set to work to devise improved and more economical methods of manufacture and to discover, if possible, new sources of supply. The needs of the moment were urgent and the Committee devoted itself mainly to the above-mentioned empirical enquiries which promised early practical results rather than to more fundamental and systematic investigations, although the latter would, in the end, be of more lasting value. At the outset, the four following lines of enquiry were adopted :—

- (a) An attempt to evolve physical and mechanical glue tests (to supplement those of the Aircraft Inspection Department whose immediate object was to enable bad glues to be condemned) such as would differentiate between samples of tolerable merit. In this way it was hoped to obtain a closer knowledge of the characteristics of good adhesives, cements and glues for wood.
- (b) To ascertain to what substance or substances, and to what properties of such substances, the adhesive strength of the gelatin base glues is due.
- (c) To discover whether and where wastage of raw material occurred in the course of the existing manufacturing processes, and if so, to suggest a remedy.
- (d) To examine, from the point of view of their adhesive power, the physical properties of substances obtainable from sources other than bone, hide or casein, and if possible from waste products of which supplies existed in this country.

4. For several months the work of the Committee proceeded along these lines and a number of useful results were obtained and are described in the following pages. Upon the cessation of hostilities, however, the more empirical enquiries which had been undertaken were no longer of the same premier importance, and it became possible for the attention of the Committee to be directed to more fundamental problems, on the solution of which real advance in the knowledge and use of adhesives depends. The experience already gained had shown that the need for a systematic investigation of the general problems of adhesives was very great and the results and experience already obtained gave hope of success. The question of finance, however, threatened to put a stop to any further work. By June, 1919, the sum of money placed at the disposal of the Committee by the Ministry of Munitions would be practically exhausted, and the Ministry did not repeat the grant. In May of that year the Committee consequently requested the Department of Scientific and Industrial Research to assist in the continuation of the work or alternatively to assume responsibility for its future conduct.

5. Adhesives are materials of very widespread application in industry; practically every branch of manufacture utilises them

in some form at some stage. It was to be hoped that researches of the kind contemplated would be financed, in part at least, by contributions from industrial firms, and that it would be possible to form a Research Association with this end in view. An examination of the position by the Department, however, showed that this was, for a time at least, impossible. In the first place, the manufacture of adhesives is only rarely the chief activity of an industrial concern; more frequently products other than adhesives form the principal output of the firm, whose interest in research upon the subject is correspondingly restricted. Secondly, the investigation of adhesives must be undertaken from the point of view of the consumer as well as of producer; to a certain extent the two interests are necessarily conflicting, and experience has shown that it is most difficult to reconcile them within one organisation representative of both industrial functions. Finally, the most diverse origin of the adhesives commonly used, and the great variety of uses to which they are put in industry, in themselves constitute difficult obstacles to the formation of a representative research organisation. For such reasons as these it was found impossible, at the time, to form a Research Association or similar body in which industry should have an immediate controlling interest. The Advisory Council of the Department recognised, however, the scientific and industrial value of investigations such as were contemplated, and it was decided to make arrangements for the continuation of the work. The investigations of the Committee were taken over by the Department, and a new small committee—the Adhesives Research Committee—was appointed to direct the work. This arrangement had the advantage that, in the event of it being found possible subsequently to form an Adhesives Research Association, the transfer of the investigations and accumulated results would be a comparatively simple matter.

6. The new Committee, which was appointed in December, 1919, is now constituted as follows :—\*

Lieut.-Colonel Mervyn O'Gorman, C.B. (*Chairman*).

Sir W. M. Bayliss, F.R.S.

Professor J. B. Farmer, F.R.S.

Sir Herbert Jackson, K.B.E., F.R.S.

Professor J. W. McBain, M.A., Ph.D.

Professor W. W. Watts, F.R.S.

Mr. F. E. Smith, O.B.E., F.R.S., Director of Scientific Research, Admiralty.

Mr. G. Rotter, O.B.E., D.Sc. (War Office).

Mr. J. E. Ramsbottom, D.Sc., Ph.D. (Air Ministry).

7. The new Committee has continued certain of the investigations initiated by the Conjoint Board Committee, and, in addition, has undertaken fresh researches of a more fundamental

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\* After the report had been completed, Dr. T. Slater Price, O.B.E., was appointed a member of the Committee.

character. Thus considerable progress has been made in the development of vegetable adhesives and in their industrial applications. Much work has also been done upon the chemistry of gelatin and gelatin glues, and, in particular, an attempt has been made to discover the nature of the changes occurring during the formation of gelatin from its praecursors in animal tissues.

8. In the manufacture of glue and other adhesives there are very wide variations in the processes employed by different firms and in the character of their finished products. It would seem, indeed, that with few exceptions the manufacturing processes employed in this country are purely empirical and without any real scientific basis. From this frequently follows an output of lower yield and grade than should be the case. It is hoped that the investigations of the Committee will be of assistance in the evolution of a sound technology of glue manufacture. There exists a prejudice—perhaps not altogether unwarranted by experience in the past—against adhesives, other than the common gums, derived from vegetable sources. It seems by no means improbable that valuable adhesives should be obtainable from such sources, and this expectation is strengthened by the results which have so far been gathered by the Committee.

9. Finally, it may be added, there is still no generally acceptable explanation of the action by which glues, etc., cause surfaces to stick together. It is obviously necessary to investigate the nature of adhesion, be it physical or chemical. This problem, which has such an important bearing upon many branches of industry, is constantly before the Committee.

## II.—<sup>c</sup>MECHANICAL TESTS OF ADHESIVES FOR TIMBER

10. An examination of the scattered literature of the subject quickly shows the unsatisfactory state of affairs as regards the testing of adhesives by means of mechanical tests of cemented wood joints. There is no standardised method of carrying out such tests, and it is evident that much investigation must be undertaken before there can be adopted any generally acceptable standardised procedure. Thus, among the variable factors in such tests are the nature of the tests themselves (whether tension, shear or impact), the nature and condition of the wood used, the method of preparing the adhesive and the wood for the test joints, and the form and size of joint employed in making up the test piece.

11. No really satisfactory methods of testing glues, etc., mechanically, otherwise than by means of wood joints, appear to have been devised. Weidenbusch (1859) employed rods made up of plaster of Paris and glue, but the results appear to be uncertain. Gill (Journ. Ind. Eng. Chem., 1915, **7**, 102) used briquettes made up of fuller's earth, diatomaceous earth, quartz sand or sawdust with various glues, but obtained similarly unsatisfactory results. The same investigator employed a modification of Setterberg's method (Schwed. Tech. Tidskrift, **28**, 52) in which the strength of glued paper is measured; the results of this test are of qualitative, rather than quantitative, significance. Gill (*loc. cit.*) has also used porcelain, glass and tiling in the making of glued test joints, but has found them all unsatisfactory. Hopp (Journ. Indust. Eng. Chem., 1920, **12**, 356) used strips of dried glue ground to standard size, and tested them in a Schopper machine. He states that the results of his tests were concordant, but it is very doubtful whether the tensile strength of a dried sample of glue is strictly indicative of the behaviour of that sample of glue when used in making a joint between two pieces of wood in the ordinary way. It would seem to be generally agreed that in order to test the value of an adhesive for use in jointing timber, the most useful information is to be obtained from wood joints made up with that adhesive.

12. At the outset of their work it seemed to the original Committee that while the existing mechanical tests of adhesives for timber were sufficiently good to enable high-quality adhesives to be differentiated from poor ones, it would be difficult to make step-by-step improvements in good or bad glues unless mechanical tests were devised which would make it possible to distinguish with certainty between samples showing moderate differences of merit. With this object in view investigations were undertaken by Major A. Robertson, R.A.F., at the Royal Aircraft Establishment, and a report was submitted by him to the Committee early in 1919. This report has since been issued\* by

\* "Report on the Materials of Construction used in Aircraft and Aircraft Engines," Chap. XI, page 132. Published by H.M. Stationery Office, price 21s. net.

the Aeronautical Research Committee, and it will here suffice briefly to indicate its nature.

13. The various tests which, up to that time, had been employed for the determination of the strength of cemented joints in timber were examined and were each found to be inadequate in some particular direction. Tension tests are of two classes :—  
(a) Those of joints made along the grain; (b) those of end grain joints. In connection with the former class, two types of test piece formerly used at the Royal Aircraft Establishment were investigated and found to be unsatisfactory on account of the unequal distribution of stress produced by the bending of the specimen when loaded. Test pieces of two other designs (described in the report) were examined and found to be satisfactory in securing a reasonably good stress distribution. It is interesting here to note that from some tension tests on a very small piece of glue (turned up from a piece of cake glue) the tension stress of the glue itself appeared to be at least 3,000 lbs. per square inch, *i.e.*, more than twice what is obtainable normally between glue and wood. As regards the second class of joints—end grain joints—a convenient form of test piece is described in the report. Preliminary tests on this type gave promising results, and it was found that good results could be obtained with soft timbers as well as with hard. Reference is also made in the report to the Spandau test (used in Germany), which is a modification of the direct tension test; in this test the pieces are glued end grain to end grain and the specimen is broken by bending. An advantage of the Spandau test piece is that this can be used for an impact test which it may ultimately be desirable to introduce.

14. Major Robertson's report further deals with shear tests, and an examination is made of the various types of test pieces which have been used by different experimenters. It is shown that the majority of these are unsatisfactory for in none is the stress on the joint a simple shear. It is suggested that the easiest method of carrying out a shear test is to make the joint inclined at about 15 or 20 degrees to the axis of a tension test specimen (figured in the report). The stresses in the joint are then a shear and a tension and, if the angle is between the limits given above, the stress causing failure will generally be the shear stress.

15. The report concludes with the outline of a procedure for testing adhesives, based on the experience gained in the work mentioned. The above gives the barest synopsis of the report, which should be consulted in its original form by those interested in strength tests of adhesives for timber.

16. Since the issue of Major Robertson's report, the attention of the Committee has been directed to other investigations, and the report consequently represents the stage to which the enquiry has been brought; it is hoped to return to the subject at a later date.

### III.—CASEIN ADHESIVES

#### GENERAL

17. The casein cements possess certain marked advantages over gelatin glues. Their adhesive powers are nearly equal to those of the best gelatin glues. They are easily rendered fluid and keep so for a comparatively long time. They can be used cold, are inodorous, are not inclined to foam and can be ready for immediate use since they do not require soaking or heating. Once dry they are not affected by moisture and they are very resistant to bacterial action. These advantages rendered them useful for aeroplane construction and the consumption of casein during the war was consequently high. It was, in fact, feared that the use of casein for this purpose might ultimately be restricted by the need for economy in foodstuffs. The processes for the manufacture of casein in this country in the early stages of the war were found, moreover, to be wasteful. In one stage of the manufacture alone—the milling process—the losses were stated in some instances to be of the order of 60 per cent. of the total weight of casein subjected to grinding.

18. A marked disadvantage of the commercial casein at this time was its lack of uniformity. So wide were the variations between lots of casein from different makers, and even between different consignments from the same maker, that it was necessary to test the suitability of every consignment separately and to vary the formula for the adhesive appropriately. For these reasons it seemed to the original Committee to be necessary to investigate the processes for the manufacture of casein with a view to elaborating one which would yield a product of satisfactorily uniform properties, with a minimum of waste. It seemed possible, further, that, could such a process be devised, it might also be applied to the production of other proteins equally suitable for the preparation of adhesives.

19. The casein used in adhesives is the protein precipitated from 'skimmed milk by the action of rennet or of acids; the precipitated material is washed, dried, and finely powdered. The lack of uniformity in samples of technical casein is doubtless due in part to the variable conditions under which it is precipitated. Attention has recently been drawn to this point by Mansfield Clark, Zoller, Dahlberg and Weimar (*Jour. Indust. Eng. Chem.* 1920, **12**, 1163), who recommend the use of hydrochloric acid: sulphuric acid is objectionable if the bye-product lactose is to be isolated from the liquor remaining after precipitation of the casein. Clark and his collaborators recommend the use of hydrochloric acid of a fixed strength (which he has ascertained by experiment) for precipitating the casein, and for washing the latter they use water which has been acidified to the same degree. The precipitation must be carried out at a fixed temperature. This procedure, followed by prescribed processes of grinding and drying, leads they state to a product remarkably

uniform in its chemical and physical properties. The Committee have had no opportunity of testing the methods recommended by these workers, but they are of opinion that, on scientific grounds, these methods are well worthy of consideration by casein makers in this country.

20. The drying stage in the manufacture of casein is one which appeared to the original Committee of the Conjoint Board to be capable of much improvement, and investigations were undertaken with this end in view. In the ordinary course of manufacture the precipitated casein, after washing and drying, is obtained in horny masses and, before being used in adhesives, these are milled so as to pass through a 60-mesh sieve. The milling entails a considerable loss; moist grinding cannot be employed since the moist powder, on drying, is again converted into hard lumps. The method of drying casein which was elaborated as the outcome of experimental work may briefly be described as follows; the method was not carried beyond a laboratory scale. The precipitated and washed casein, after drying on cloths, is ground in a mortar. A homogeneous paste is made with water and ammonia, the latter being added until the mixture smells faintly of the reagent. The alkaline paste is then spread in thin layers on glass plates which have previously been given a coating of paraffin oil as thin as possible. The plates are then exposed to a current of air at approximately 37° C. until the casein is dry. The casein, possibly in the state of an ammonium derivative, is thus obtained in the form of thin scales which can readily be peeled off the plates and ground to a fine powder. Sodium hydroxide may also be used in this preparation; the alkali is added until a faint pink colour is obtained upon the addition of phenolphthalein to a small quantity of the mixture; finally ammonia is added to prevent hydrolytic dissociation of the sodium salt during drying. The above method proved to be useful in yielding a product which presented no difficulties in grinding. At this stage of the investigation it was found that the method had been anticipated and further development was left to private enterprise.

21. Still other factors influence the uniformity of commercial casein. These are the presence of fat, the presence of traces of acid and lastly the state of division of the substance. A few experiments were made to ascertain the influence of these factors upon the value of casein as a constituent of adhesives, with the results given below. (For these tests a simple casein-borax cement was used containing 15 to 20 parts of casein and 6 to 10 parts of borax.)

(a) *Influence of Fat.*

- (i) The strength of the cement as measured by the Aircraft Inspection Departments' tests, diminished markedly with increase of the fat content.

- (ii) The shear strength for plywood similarly diminished with increase of fat.
- (iii) The waterproofness is not appreciably affected by moderate amounts of fat.

(b) *Influence of Acidity.*

- (i) The strength of the cement (A.I.D. tests) decreases as the quantity of acid present increases. Other experimenters have obtained similar results.
- (ii) The quantity of water necessary to obtain a cement of suitable consistency increases with increasing quantity of acid left in the casein.
- (iii) If an excessive amount of acid be present it is impossible to obtain the casein free from lumps.

These results are such as would be anticipated from a consideration of the chemical reactions taking place. Free mineral acid attacks casein giving gelatinous products in the first place; the latter form a coating round more casein and thus lead to "lumpiness." The casein is contaminated with organic impurities affecting its physical properties, while other new factors are introduced owing to the liberation of boric, silicic acids, etc., by the simultaneous attack of the original free acid upon the borax, sodium silicate, etc., used in the cement.

(c) *Influence of Fineness.*

Mixtures prepared by using 30, 60 and 90-mesh casein gave results indicating increase of strength with increasing fineness. It was found, however, that if the 30-mesh casein was left to soak for some time the resulting cement approximated in strength to that prepared from 90-mesh casein. This would appear to indicate that the best results are to be obtained from casein in a form in which it most readily reacts with the remaining compounds of the cement.

## T COMPOSITION OF CASEIN CEMENTS.

22. Many formulae have been employed in the production of casein cements. The attention of the original Committee was drawn to one of them, adopted by the Air Board (Specification V/2, February, 1918), which was the outcome of investigations carried out at the laboratories of the Aeronautical Inspection Department. Cement made to this formula gave excellent results when fresh. It was found, however, that the keeping properties of the cement were poor and that, especially in tropical climates, the strength diminished as time went on. This change was due to the presence of excess of caustic soda. At the request of the Committee further experiments were carried out by the Aeronautical Inspection Department for the purpose of devising a

cement which would have good keeping qualities as well as good adhesive properties. As the result of a large number of trials the following formula was evolved :—

|                      |     |     |     | Per cent. |
|----------------------|-----|-----|-----|-----------|
| Casein...            | ... | ... | ... | 78        |
| Dry sodium carbonate | ... | ... | ... | 45        |
| Sodium fluoride      | ... | ... | ... | 40        |
| Freshly slaked lime  | ... | ... | ... | 12·5      |
| Sodium arsenate      | ... | ... | ... | 1·0       |

All ingredients must be ground to pass a 90-mesh sieve.

23. For use, this cement may be mixed with (a) water, and (b) a weak solution of gelatin glue. Joints made up from the cement in each way were tested in comparison with an American casein cement\* by the A.I.D. methods with the following results :—

*Breaking Stress in Pounds per Square Inch.†*

| American Cement. | (a)         | (b)         |
|------------------|-------------|-------------|
| • 1,029          | ..... 910   | ..... 1,010 |
| 1,019            | ..... 1,110 | ..... 950   |
| 1,034            | ..... 1,065 | ..... 1,105 |
| 1,322            | ..... 960   | ..... 1,140 |
| 1,146            | ..... 925   | ..... 1,090 |
| 1,160            | ..... 780‡  | ..... 935   |
| 1,058            | ..... 885   | ..... 1,015 |
| 907              | ..... 790   | ..... 885‡  |
| 1,055            | ..... 910   | ..... 1,210 |
| 945              | ..... 1,040 | ..... 1,030 |
| Average          | 1,067       | 955         |
|                  | _____       | _____       |
|                  |             | 1,054       |

24. In addition to the joints required for the above tests a number of others were made up at the same time for use in investigating the effects of dry heat, moist heat and weathering on the strength of the new cement.

*Dry Heat.*—The joints were kept for eight days at 45°C. with two interruptions of 24 hours each. The figures given below represent the average of five experiments, in each case, to determine the breaking stress.

| American Cement. | (a) | (b)  | Airscrew † Glue. |
|------------------|-----|------|------------------|
| 880              | 825 | 1025 | 987·5            |

\* The composition of the American cement is approximately :—

|                  |     |     |     |     |              |
|------------------|-----|-----|-----|-----|--------------|
| Lactic casein    | ... | ... | ... | ... | 75 per cent. |
| Agar-agar        | ... | ... | ... | ... | 5 " "        |
| Sodium carbonate | ... | ... | ... | 4   | " "          |
| Sodium fluoride  | ... | ... | ... | 4   | " "          |
| Slaked lime      | ... | ... | ... | 12  | " "          |

† The average strength of similar joints made from good airscrew glue is 1,100 lbs. per square inch.

‡ Values excluded in calculating averages.

*Moist Heat.*—The joints were here kept for eight days in a moisture saturated atmosphere at approximately 40°C.

| American Cement. | (a)  | (b)  | Airscrew Glue. |
|------------------|------|------|----------------|
| 1213             | 1082 | 1175 | 1094           |

*Weathering.*—The joints were exposed to changeable atmospheric conditions (October) for 15 days without protection.

| American Cement. | (a) | (b) | Airscrew Glue. |
|------------------|-----|-----|----------------|
| 867              | 947 | 970 | 555            |

25. The results briefly reported above show that a cement prepared according to the formula given has adhesive properties and keeping qualities nearly as good as—and in some cases superior to—the imported American cement.

26. One further point remains for consideration, namely, the susceptibility or otherwise of the cement to bacterial action which destroys the adhesive properties. The resistance of the new cement to bacterial action was found to be very good, as will be seen by reference to the results of the tests carried out by Mr. C. J. Wilson, of the Royal Aircraft Establishment.

The cements tested were :—

1. An American cement.
2. A. } The new cement prepared as described under (a) and
3. B. } (b) above.
4. Cement prepared as in 3 but using lactic casein.

Portions of these were kept in an incubator at 35°C. in which the air was saturated with moisture. On microscopic examination—

1. contained many organisms, mostly quiescent, but some displaying movements which were not pedetic;
2. very few organisms and no sign of life;
3. very few organisms and no sign of life;
4. more organisms than in 2 and 3 but fewer than in 1; a few of them were mobile.

Next, scrapings of the cements were placed in equal quantities of water and kept in an incubator for 24 hours :—

1. contained a good many bacilli, some of which were certainly alive;
2. swarming with bacilli, the greater number of them in a very active state;
3. comparatively few organisms and only motion visible due to currents;
4. as in 2, but the organisms were less active, a considerable proportion of them being quiescent.

27. Perusal of this section of the report will show that, while several promising results have been obtained, much work still remains to be done upon casein adhesives. The work has so far been, to a certain extent, of an exploratory rather than of a systematic character, and the Committee purpose to return to it at a later stage.

#### IV.—GELATIN ADHESIVES

28. The gelatin adhesives, commercially known as glues, are the materials obtained by extracting certain animal tissues, chiefly bones, skins, cartilage and tendons, with boiling water. These glues are usually regarded as impure gelatins containing varying amounts of the decomposition products of the latter, and for the immediate purpose of this report, the above description may be adopted. More detailed reference will be made later, however, to the composition of glue and gelatin. At present little is known as to the relationship between the chemical composition and the physical properties of glues, and there is certainly no generally acceptable chemical criterion of the adhesive quality of a glue. The glue-making industry is based very largely, if not mainly, upon empirical methods evolved out of long experience, and under these conditions real advance in the industry is very slow.

29. A further obstacle to progress in the industry lies in the peculiar demands of the consumers. Many trades each require a special glue of their own, made from particular raw materials by specified processes. There is consequently a tendency for each manufacturer to produce special "lines" which may or may not be of service to the bulk of the consumers.

#### GENERAL LINES OF INVESTIGATION

30. During the war a rapidly growing demand arose for the production of glues of high adhesive power, and it was found very difficult to obtain regular supplies of glue of consistently good, specified quality. In other words, the methods employed in the industry could not be relied upon to give an uniformly good product. It was clear that much investigation, including a certain amount of fundamental research, was needed if uniformity and high quality of output was to be secured. As a preliminary to their work the original Committee visited several glue factories and examined the processes there in operation. It is unnecessary here to describe these processes; a succinct, general account of glue manufacture will be found in the "Report on the Materials of Construction used in Aircraft and Aircraft Engines," published by H.M. Stationery Office.

31. As a result of their preliminary enquiries the Committee decided to undertake investigations of the following nature :—

(i.) A detailed examination of gelatin adhesives, with a view to discovering to what glues owe their adhesive properties.

This involves :—

(a) an inquiry into the physical state of the gelatin aggregate and its relation to the adhesive properties of glues, and the action of salts upon the aggregate;

(b) an examination of the relationship between the chemical properties of glues and their adhesive strengths for the purpose of developing chemical tests by which the adhesive quality of glues may be estimated.

(ii.) An investigation of the effect of various preliminary treatments during the preparation of a standard gelatin from raw materials, including an examination of the effect of heat upon gelatin and its solutions.

32. Work of this nature was commenced by the original Committee and continued by the present one; the enquiry is still in progress. The various investigations are not described here in the chronological order in which they were undertaken, nor are the results obtained by the original Committee separated from those of the present one. It has been thought better, for the sake of continuity and ease of reference, to group together the results of investigations of cognate subjects.

#### GENERAL NATURE OF THE RESULTS OBTAINED

33. Before giving a detailed account of the experimental work upon glue and gelatin, it may be of advantage to describe in general terms the character of the work and the nature of the results.

34. Glues are most commonly employed for sticking together surfaces of wood; these are relatively rough and uneven, and no matter how closely a pair of wood surfaces be pressed together the actual points of contact form probably but a fraction of the area of overlap. Between the points of contact are relatively large air spaces. In a well glued joint, the glue not only covers the portions of the two pieces of wood which touch, but it fills in the spaces which would otherwise be occupied by air. In this way only can a strong rigid structure be obtained. It is possible to grind and finish two surfaces of a material such as glass, with such accuracy that they fit one another almost exactly. Thus two lenses can be ground with the same radius of curvature, and the surfaces polished until they are extremely smooth. If one such lens has a convex surface and the other a concave, and these two surfaces are pressed together, it is found that without an adhesive they adhere so tightly that considerable force may be required to separate them. In such a case the two surfaces are so highly finished—smoothed—that large areas of them are in actual contact. The state of affairs is very different with wood surfaces, where the interspaces must be filled with an adhesive. For this purpose an adhesive must obviously be, at the time of application, a viscous liquid, since a mobile one could not be retained in place, as it were, until the joint was made.

35. If, however, the glue is too viscous, it will not readily fill the interspaces, and the joint is consequently weakened. Thus for each class of material to be glued there is possibly an

optimum viscosity of glue dependent upon the size of the inter-spaces and pores of that particular material. This may be one underlying reason for a characteristic feature of the glue industry, *i.e.*, the selection of numerous special glues for specific purposes.

36. Adhesives, then, must be viscous substances; since viscosity is generally connected with high molecular weight, or at least with high molecular association, they are usually substances of complex chemical composition. It is only reasonable to suppose that if the complex adhesive is broken down into simpler chemical units its adhesive strength will suffer. This is not to imply that a body of complex physical structure is necessarily a better adhesive than a body of a simpler physical structure. Gelatin, the basis of glues, is a substance of complex molecular composition which is readily broken up into simpler bodies such as amino acids. This decomposition is brought about by dilute acids or alkalis, and possibly even by water alone under certain conditions. For this reason, prolonged heating with water, or relatively short heating with acids or alkalis, tends to break up gelatin, and consequently glue, and poor gels are the result. While the gel-forming power of a glue is not an absolute criterion of its value, yet it is generally the case that a gelatin or glue which forms a poor gel is not a good adhesive. The detrimental effects of water, acids and alkalis upon glue are in the order named, alkalis being much more destructive than the other two. In the preparation of glue from bones, hides, etc., the extracting liquid should be as nearly neutral as possible (special care being taken to avoid the presence of free alkali), and the period of heating should be as brief as possible, having due regard to other conditions.

37. As an extension of the work relating to the determination of the most suitable conditions for the extraction of glue, an attempt has been made to ascertain the nature of the reactions taking place during the production of glue, or rather of gelatin, from bones, tendons and skins. The evidence seems to show that these animal tissues contain substances known as ossein and collagen which combine with water to form gelatin. Thus, during the extraction of glue or gelatin, two reactions may take place :—

- (a) hydrolysis of ossein or collagen to form gelatin;
- (b) hydrolysis of gelatin giving harmful degradation products.

Fortunately the first reaction appears to proceed much more rapidly than the second, but the existence of the latter must be borne in mind. Its progress will be minimised by carrying out the extraction of bones, etc., as rapidly and at as low temperatures as possible consistently with economic working.

38. The extraction of tendons and skins is carried out technically at comparatively low temperatures but, when undecalcified bones are used, higher temperatures are employed and

poorer glues, as a rule, result. It is here shown that if the bones are ground to a specified size before digestion with water the temperature can be lowered and the time of heating reduced, the product being a good, clean *gelatin*.

39. The general conclusions to be drawn from these investigations may be summarised thus :—

- (i.) Excessive heating of glue or gelatin solutions is to be avoided. The concentration of gelatin solutions at relatively low temperatures *in vacuo* yields a superior product.
- (ii.) Digesting liquids should be as nearly neutral as possible.
- (iii.) In cleaning skins and tendons preparatory to use in glue manufacture the strength of the alkali solution used should be less than 2 per cent.
- (iv.) A gelatin can be obtained from ground undecalcified bones (1/32nd inch diameter) by digestion at a temperature not exceeding 100° C.
- (v.) Acids or alkalis used for the preparation of bones, tendons and skins must be removed as completely as possible before digestion. The removal is best effected by washing with (a) water, (b) weak acid or alkali to neutralise any residual alkali or acid, and finally with (c) water once more.

#### THE CLASSIFICATION OF GLUES

40. In industrial practice it is, of course, of great advantage if the quality of a raw material can be ascertained by means of a single test. While many methods have been applied to the examination of glues with a view to their classification, the general opinion is that no single test can be employed for this purpose. Among the numerous tests proposed from time to time are the following (*see* Allen's "Commercial Organic Analysis," Vol. 8, page 605) :—

- (i.) *Mechanical Tests of Glued Joints.*
- (ii.) *Acidity.*—This can be determined qualitatively by litmus paper, or quantitatively (*see* Kissling, *Chem. Zeit.* 1887, **11**, 691 and 719; Kalmann, *Journ. Soc. Chem. Ind.*, 1890, **9**, 113).
- (iii.) *Viscosity.*—This can be ascertained roughly by measuring the time taken by a fixed quantity of glue solution of standard strength, at 80° C., to run out of a "standard" pipette. More refined methods and apparatus have been devised for this purpose. *See*, for instance, Engler, *Journ. Soc. Chem. Ind.* 1890, **9**, 654 and Rideal and Slotte, *ibid.* 1891, **10**, 615. Some investigators use the viscometer devised by Scarpa.
- (iv.) *Odour.*—The decomposition of glue is readily detected by smell unless this be masked by antiseptics or ethereal oils.

(v.) *Grease*.—Grease is a most deleterious impurity in glue and this is especially the case when the latter is employed in sizing. The amount of grease can be roughly estimated by mixing a brushful of glue solution with an aniline colour and painting with it a piece of white paper; the number of "eyes" produced is roughly proportional to the amount of grease in the glue. (See Bailey, Conn. Ag. Stat. Bull. **219**, 213.)

(vi.) "*Foam*."—The absence of "foaming" is especially essential in veneering and where glueing is performed by machinery. The glue solution of definite strength is shaken in a tube of standard dimensions for a definite time and the volume of the resulting foam is compared with that produced by standard glues treated in a similar manner.

The cause of "foaming" has been investigated by Trotman and Hackford (Journ. Soc. Chem. Ind. 1906, **25**, 104) and by Watson (*ibid.* 209). Rideal has suggested that "foaming" is due to the presence of mucins.

(vii.) *Setting Time*.—The time taken by a glue to set to a jelly under standard conditions is compared with that taken by standard glues.

(viii.) *Jelly Strength or Finger Test*.—In this test the glue solutions are arranged in order of merit by comparing the strength or resiliencies of their jellies as ascertained by the pressure of the finger tips. While the personal factor in this test is obviously a very important one, it is possible for expert operators to obtain fairly consistent results with it.

The testing of the comparative consistencies of jellies by measuring their capacities for bearing weights was originally suggested by Lipowitz and apparatus for this purpose has been devised by, among others, E. S. Smith (United States Patent 911,277) and by Alexander (United States Patent 882,731), who tests jellies unsupported by containing vessels.

(ix.) *Keeping Quality*.—Jellies of suitable strength made from the glues under test are allowed to stand for some time under conditions as nearly as possible identical with those under which the glues will be used; observation from time to time indicates the probable stability of the glues.

(x.) *Melting Point*.—Methods for determining the melting points of glues have been proposed by Chercheffsky (Chem. Zeit. 1901, **25**, 413) and Cambon (Journ. Soc. Chem. Ind. 1907, **26**, 703).

(xi.) *Water Absorption Test*.—The weight of water absorbed by a known weight of the glue when immersed in water at room temperature for 24 hours is measured. High quality glues absorb about 10 to 15 times their weight of water, and medium glues 3 to 5 times their weight while low class glues form a sludge under these conditions.

(xii.) *Estimation of Non-Gelatinous Constituents*.—By the addition of alcohol to a glue solution all the gelatinous material can be precipitated, the non-gelatinous materials

remaining in solution. Stelling (Chem. Zeit. 1896, **20**, 461) suggests this as the basis of a method for estimating the non-gelatinous content of a glue.

41. The original Committee found it necessary to re-investigate certain of these tests and the present Committee have continued the work; the results are given below.

(a) *Mechanical Tests of Glued Wood Joints.*

42. Reference has already been made to the various strength tests of glued joints which have been used or suggested from time to time. The chief mechanical test employed in the work of the Committee was a simple tensile test evolved by the workers at the Aeronautical Inspection Department. This test was found to be the most reliable and suitable having regard to speed and relative accuracy; a description of the technique of the test may be of utility.

- (i) The test pieces in the earlier work consisted of carefully selected pieces of hard, dry, American, straight-grained walnut, two inches wide, nine inches long and three-eighths of an inch thick. The flat sides are planed true and are toothed with a fine toothed plane (25 teeth per inch).
- (ii) The glue is soaked in the requisite amount of water at room temperature for 24 hours after which it is heated between 60 and 80°C. for a short period and allowed to cool to 60° before application.
- (iii) Before making test joints the wood is allowed to remain for several hours in a constant temperature oven at 35°C.
- (iv) Two pieces of warm wood are then removed from the oven and the glue is quickly applied with the fingers to one surface of each test piece; the pieces are then placed together in such a manner that a simple one-inch overlap is obtained, giving a joint with two square inches of glued area. During the application of the glue care must be taken to avoid the formation of air bubbles.
- (v) The joint is then secured in a wooden frame with the aid of an iron clamp. By means of tested box-springs a standard pressure of 400 lbs. is applied for 12 to 18 hours.
- (vi) The joint is unclamped at the end of this time and allowed to stand for three days. Its breaking stress is then determined in a form of Avery or Buckton cement-testing machine adapted to the testing of wood joints, the usual precautions being taken. In a more recent type of Avery machine, shorter pieces of wood (only 4½ inches in length) are employed.

43. At least four test joints should be made from each sample of glue and the mean breaking stress of the four joints determined. This test cannot in itself be regarded as an absolute criterion of

the value of a glue, since a number of disturbing factors arise. These include :—

- (a) variations in porosity of the wood.
- (b) variations in the heating of the glue, and in the temperature of the wood;
- (c) variations in the thickness of glue films due to differences in the viscosities of the glues;
- (d) changes in the humidity and temperature of the atmosphere;
- (e) irregular application of the load during testing.

44. If, however, care is taken to keep these factors in mind, to make experiments under conditions as uniform as possible, and to eliminate isolated results so irregular as obviously to be due to mechanical defects, the experimental error will generally not exceed 10 per cent.

45. The following standards for glues have been fixed by the British Engineering Standards Association. These standards are adopted for  $4\frac{1}{2}$ -inch test pieces.

| <i>Class.</i>      | <i>Breaking Stress.</i> | <i>Use.</i>                           |
|--------------------|-------------------------|---------------------------------------|
| Propeller glues... | 1,100 lbs. per sq. inch | Airscrew manufacture.                 |
| Class I ... ...    | 1,000     "     "       | Important stress-bearing work.        |
| Class II     ...   | 900     "     "         | No stress-bearing work.<br>and under. |

(b) *The Setting Times of Glues.*

46. The setting time of a glue has been found to furnish a satisfactory indication of its behaviour in use, and the following simple and reliable technique of the test has been adopted. A 10 per cent. solution of the glue is prepared by soaking 5 grams of the glue overnight in 50 cc. of water. The soaked glue and supernatant liquid are then heated together in an incubator at 37°C. and when complete solution has been obtained samples are poured into a series of glass cylinders of standard size (0.5 cm. diameter and 8 cm. long); the vessels are at once immersed in a thermostat maintained at 20°C.

47. Every few seconds a steel ball of standard size and weight is cautiously dropped into a tube of glue solution until finally a ball is unable to sink more than half way through the jelly. When near the setting point the balls will fall only slowly through the liquid, which is gradually becoming a gel. At this stage successive balls should not be dropped into the same tube; balls should be dropped each into a fresh tube at short intervals. The time from the removal of the tubes from the thermostat to the setting of the jelly, as indicated above, is noted on a stop-watch; this is termed the setting time. After a little practice it can be determined with a fair degree of accuracy.

(c) *The Melting Point of a Glue.*

48. The melting point of a substance such as glue is, of course, somewhat difficult to ascertain with any degree of accuracy. Several methods have been suggested and advocated by different

workers. In the present investigations it was found convenient to determine the melting point of a 10 per cent. solution of the glue in the following manner.

49. A 10 per cent. solution of the glue is prepared in the same way as in (b) above and poured into a tube similar to those described in the same test until the tube is about two-thirds full. After the jelly has been allowed thoroughly to set, a standard steel ball of as large diameter as possible is cautiously placed upon the surface of the jelly. The tube is then fastened to a thermometer and lowered into a beaker of water until the water reaches nearly to the top of the tube. The water is then gradually heated with constant stirring, and at the moment when the ball commences to break the surface of the jelly the temperature is read off. This temperature is taken as the melting point of the 10 per cent. solution of the sample and is considered to be characteristic of the glue in question. This method has been found, in practice, to be the simplest and most consistent.

(d) *Diffusible Nitrogen Test.*

50. In the course of the work of the Committee a new method of testing glues has been evolved and to it may be given the above title. The test supplies an indication of the stability of the glue towards water, and the results of the test when applied to different glues furnish a rough measure of their tensile strengths, the stronger glues having generally a low diffusible nitrogen. This is not, however, universally the case. Glues of good adhesive strength have been obtained that yield jellies, when prepared according to the specification in this section, which, on application of the Diffusible Nitrogen Test, pass entirely into solution.

51. The glue under examination is made up into a jelly with water in such proportions that 21 grams of nitrogen are contained in 75 c.c. of water; approximately 15 grams of glue is required. The correct amount of glue is weighed out and to it is added 75 c.c. of water. The mixture is allowed to stand over night to enable the glue to be thoroughly swollen. It is then heated to 37°C. for two hours and finally to about 90°C. for 30 minutes, and subsequently poured into a Petri dish (14 cms. in diameter) and allowed to stand until set. Water (100 c.c.) is then poured over the surface of the jelly and the dish is now placed in a thermostat at 20°C. for 20 hours. At the end of this time the aqueous phase is poured off and the nitrogen content in 100 c.c. of the solution is determined by Kjeldahl's method. The results are expressed in milligrams of nitrogen per 100 c.c. of aqueous phase.

52. The results tabulated below serve to show the relationship existing between the amount of diffusible nitrogen and the tensile strength; the latter is roughly inversely proportional to the former

*Diffusible Nitrogen and Tensile Strength of Glues*

| Sample.   | Diffusible Nitrogen in milligrams per 100 c.c. of aqueous phase. | Tensile Strength in lbs. per sq. in. [1 part glue to 2 pts. water]. |
|-----------|--|---|
| Hide Glue | No. 8 ... 54   | 1,390   |
|           | No. 6 ... 73   | 1,390   |
|           | No. 7 ... 82   | 1,355   |
|           | No. 4 ... 72   | 1,345   |
|           | No. 3 ... 121  | 1,275   |
|           | No. 5 ... 141  | 1,190   |
|           | No. 9 ... 118  | 1,120   |
|           | No. 1 ... 121  | 1,070   |
|           | No. 12 ... 252   | 862   |
|           | No. 11 ... 241   | 672   |
|           | No. 17 ... 13  | 1,410   |
| Bone Glue | No. 16 ... 23  | 1,210   |
|           | No. 13 ... No separation of phases.                              | 851   |
|           | No. 14 ... " " "   | 829   |
|           | No. 15 ... " " "   | 437   |

In the course of the researches, gelatins were prepared from different sources and under varying conditions, with the object of ascertaining the degree of variation in the above-mentioned factors and to correlate it, if possible, with the origin and history of the glues.

Some of the results are given in the following table:—

| Sample.                         | Tensile Strength in lbs. per sq. inch [1 pt. glue to 3 pts. water.] | Diffusible Nitrogen in milligrams per 100 c.c. of aqueous phase. | Setting Time in seconds. |
|---------------------------------|---|--|--------------------------|
| A <sup>13</sup> ...             | 1,110   | 58   | 60                       |
| A <sup>16</sup> ...             | 1,100   | 50   | —                        |
| A <sup>14</sup> ...             | 1,090   | 83   | 45                       |
| A <sup>5</sup> ...              | 1,080   | 96   | 115                      |
| A <sup>15</sup> ...             | 1,030   | 99   | 70                       |
| A <sup>2</sup> ...              | 960   | —  | 170                      |
| A <sup>6</sup> ...              | 950   | 182  | 240                      |
| A <sup>3</sup> ...              | 930   | —  | 360                      |
| B <sup>1</sup> ...              | 1,050   | One phase  | Over 30 mins.            |
| B <sup>2</sup> ...              | 1,070   | " "  | " "                      |
| B <sup>3</sup> ...              | 650   | " "  | " "                      |
| B <sup>4</sup> ...              | 580   | " 91 "   | " 45 "                   |
| C <sup>4</sup> ...              | 1,200   | 61   | 40                       |
| C <sup>1</sup> ...              | 1,180   | 131  | 320                      |
| C <sup>3</sup> ...              | 1,130   | One phase  | 385                      |
| C <sup>5</sup> ...              | 1,040   | 139  | 230                      |
| C <sup>6</sup> ...              | 1,030   | 23   | 25                       |
| Commercial Brand Gelatin No. 1. | 1,180   | 13   | 30                       |
| Commercial Brand Gelatin No. 2. | 1,160   |  |                          |

A. Decalcified bones.

B. Undecalcified bones.

C. Cartilages.

53. So far the method may be said to have yielded satisfactory quantitative indications of an important property of glues which hitherto has not been investigated, *i.e.*, resistance to water. The investigation is admittedly incomplete, and the subject requires further study. Again, the test may lend itself to the study of the size of the hydrated gelatin aggregate under various conditions. The addition to a glue solution of salts that lower the surface tension of water will tend to reduce the size of the gelatin aggregate, and presumably, consequently, to increase the amount of diffusible nitrogen. The change should be reversible, and it should be possible to trace the changes in the gelatin aggregate by ascertaining the amount of diffusible nitrogen in solution.

54. The test should further be capable of giving an indication of the change undergone by glue during prolonged heating with water. If this change consists, in part, of an hydrolysis of the gelatin with the formation of products of lower molecular complexity, the latter should be comparatively readily diffusible and capable of estimation by the test.

55. Arising out of these considerations the following investigations suggest themselves, and the Committee propose to carry them out when opportunity permits:—

- (i.) The estimation of the amount of diffusible nitrogen derived from a sample of standard gelatin in the presence of various salts, acids, and bases under prescribed conditions.
- (ii.) The estimation of the amount of diffusible nitrogen yielded by a sample of standard gelatin which has been subjected to heat in the presence of water.
- (iii.) A detailed examination of the aqueous phases containing nitrogen in the gelatin-water system for the purpose of tracing the degradation of the gelatin molecule.
- (iv.) The determination of the rate of diffusion of nitrogenous degradation products of gelatin under various conditions.
- (v.) The correlation of the adhesive strength with the alteration in gelatin corresponding to the changes in the quantities of diffusible nitrogen.

#### THE PHYSICAL STATE OF THE GELATIN AGGREGATE

56. It is obvious that the adhesive qualities of glues are related in some way to the physical state of the gelatin contained in them, and it is further evident that much useful information is to be gained by the study of the nature of the gelatin aggregate. The subject has consequently attracted the attention of the Committee and, while the work so far done is of a preliminary character only, it may be useful to set out the results at present available.

57. There can be little doubt that gelatin does not exist as such in animal tissues (bones, hides, tendons, etc.). It would seem to be present in the form of an anhydride—collagen or

ossein—but little is known of the chemical nature of the praecursors of gelatin. The work dealing with this subject is described in some detail in a later section of this report.

58. The structure of the true gelatin jelly has been the subject of much discussion. Zsigmondy ("Colloids and the Ultra-microscope") has shown that 0·1 and 0·2 per cent. solutions of gelatin are heterogeneous after they have stood for two days. This result has been confirmed by Bütschli and others whose work indicates that gelatin possesses a cellular, sponge-like structure. It should be noted, however, that a solution of gelatin prepared at the boiling point shows a homogeneous light cone in the Siedentopf-Zsigmondy ultra-microscope, and hence is presumably homogeneous. C. R. Smith (Journ. Amer. Chem. Soc. 1919, **41**, 135) has observed mutarotation in gelatin solutions, and from this phenomenon argues the existence of two forms; one (gelatin A) is, he states, stable above 35° C., while the other (gelatin B) is stable below 15° C. Similarly W. Moeller (Kolloid. Zeit. 1916, **19**, 205, 213) claims that there are two forms of gelatin, e.g.,  $\alpha$  and  $\beta$  gelatins, the former constituting an irregular network, while the latter fills the interspaces. Finally, according to Weimarn (see *Grundzüge der Dispersoid Chem.* Dresden, 1911) and to Bradford (Biochem. Journ. Jan 1911), a hot gelatin solution is to be considered as not unlike a hot saturated solution of a crystalline substance. The above brief reference will serve to show that a cold solution of gelatin is to be regarded as consisting of two phases, solid and watery. This subject is dealt with more fully, and in the light of recent work upon soap systems, in Appendix II to this Report.

59. It has been stated that gelatin itself is not a good adhesive; the adhesive power of glue may be due directly or indirectly to some other product or products, and we are naturally led to consider—

- (a) What is the nature of these other products in a good glue?
- (b) What is the general state of aggregation in the hard mass of glue?
- (c) Can the state of aggregation be altered at will?

60. Investigations in connection with these three problems have been put in hand. The two first investigations are not yet sufficiently advanced for any general statements to be made; concerning the third a certain amount of information has been obtained.

#### ALTERATION OF THE SIZE OF GELATIN AGGREGATES

61. Weimarn has shown that it is possible to vary, at will, the state of aggregation of many substances, by modifying the rate of formation and the rate of growth of the particles. Thus barium sulphate has been obtained in forms ranging from a crystalline body to a jelly. If a hot gelatin solution resembles a hot saturated solution of a crystalline salt, the state assumed by the cold gelatin will depend upon (1) the rate of formation, and (2) the rate of growth of nuclei. These rates are modified by several physico-chemical factors but the influence of two of these

—surface tension and viscosity—are especially worth investigation. Schryver (Proc. Roy. Soc. 1910. B. 83. pp. 96, 113, 119) has studied the change in the state of aggregation of dispersed particles, in an aqueous medium, in relation to these two factors.

62. It was thought that the state of aggregation of gelatin might have a considerable influence on its adhesive power and accordingly some experiments were carried out with this object in view.

63. The surface-tension and viscosity of the solvent can be altered by the addition of various substances. The more the addition of a substance lowers the surface-tension of water the greater will be its dispersive power. On the other hand an increase of viscosity tends to diminish the dispersive effect. It is difficult, therefore, to determine accurately the effect of each individual factor on the dispersion of colloids. This difficulty may be overcome by choosing salts whose equimolecular solutions do not differ greatly in viscosity, but have a relatively wide range of surface-tensions; some idea of the effect of the latter factor can then be gained.

64. For this purpose a series of sodium salts of organic acids were chosen whose effects on a number of substances, existing as a dispersed phase in water, have been described in the paper quoted above.

65. The effect of these salts on the adhesive and jelly strengths of gelatin has now been ascertained.

66. In the experiments the results of which are tabulated below, gelatin solutions were made up from 10 grams of Cougnac's Gold Label gelatin and 30 c.c.s of N, N/2, N/5 and N/10 solutions of each salt, and were subsequently tested for tensile strength. A solution made from 10 grams of the same gelatin and 30 c.c.s of water was used for control purposes. The preparation of the solutions and the tests were carried out, of course, under strictly comparable conditions.

67. The jelly strengths were also tested by the method of Lipowitz. For this purpose jellies were prepared by the addition of 50 c.c.s of N, N/2, N/5 and N/10 solutions of each salt to 10 grams of the above gelatin, and a control jelly for comparison was made up from 50 c.c.s of water and 10 grams of the gelatin.

| Name of Salt.       | Normal<br>Soln. | Surface<br>Tension<br>of<br>Normal<br>Soln. | Tensile Strength expressed<br>in lbs. per sq. inch, 10 grams<br>gelatin in 30 c.c.s of :— |       |       |      | Jelly Strengths<br>5 grams gelatin in<br>50 c.c. of :— |     |     |    |
|---------------------|-----------------|---|---|-------|-------|------|--|-----|-----|----|
|                     |                 |   | N. Soln.  | N/2   | N/5   | N 10 | N. Soln.   | N/2 | N/5 | N  |
| Sodium Formate      | ...             | 1·020                                       | 1,200   | 950   | 1,000 | 960  | 55   | 55  | 55  | 55 |
| " Lactate           | ...             | 1·013                                       | 1,070   | 950   | 950   | 960  | 55   | 55  | 55  | 55 |
| " Tartrate          | ...             | 1·011                                       | 820   | 1,200 | 1,080 | 820  | 55   | 55  | 55  | 55 |
| " Citrate           | ...             | 1·016                                       | 880   | 800   | 880   | 840  | 55   | 55  | 55  | 55 |
| " Acetate           | ...             | 1·004                                       | 600   | 570   | 850   | 740  | 55   | 55  | 55  | 55 |
| " Monochlor-acetate | ...             | 1·002                                       | 840   | 920   | 870   | 860  | 55   | 55  | 55  | 55 |
| " Dichlor-acetate   | ...             | .970  | 780   | 980   | 810   | 840  | 0  | 10  | 30  | 40 |
| " Trichlor-acetate  | ...             | .905  | 840   | 890   | 930   | 880  | 0  | 15  | 30  | 40 |
| " Salicylate        | ...             | .902  | 830   | 980   | 770   | 830  | 0  | 0   | 0   | 10 |
| " Benzoate          | ...             | .897  | 520   | 620   | 760   | 770  | 10   | 25  | 35  | 45 |
| " Control           | ...             | 1·000                                       | 870   | —     | —     | —    | 55   | —   | —   | —  |

68. The effect of adding substances which increase the viscosity of solutions without appreciably altering the surface tensions was also investigated. Sugars were chosen for this purpose. The sugars were purified and molar solutions and fractions of molar solutions were used.

#### TENSILE STRENGTH

| Sugar.             | 10 grams gelatin in 30 c.c. of |           |           |           |            |
|--------------------|--------------------------------|-----------|-----------|-----------|------------|
|                    | M. Soln.                       | M/2 Soln. | M/4 Soln. | M/5 Soln. | M/10 Soln. |
| Glucose ... ...    | 940                            | 900       | —         | 810       | 910        |
| Maltose ... ...    | 990                            | 780       | —         | 1,190     | 1,090      |
| Laevulose ... ...  | 950                            | 980       | —         | 870       | 800        |
| Cane Sugar ... ... | —                              | 1,200     | 850       | 870       | 830        |
| Control (In water) | 870                            | —         | —         | —         | —          |

69. This investigation, on the action of salts is of a preliminary character only, and requires a considerable amount of further study before definite conclusions can be drawn. It was, however, undertaken at a relatively early stage of the work as it had an important bearing on the subject of liquid glues. The results quoted above require revision as certain factors have not been taken into account; thus, for example, a salt which produces great dispersion will tend to form a glue of lower viscosity than another salt of small dispersive capacity, and in making the joints more glue will be expressed the lower the viscosity, thereby weakening the joint. This factor should be taken into account in appraising the value of the results.

70. Nevertheless it is obvious that certain substances have a marked effect on the adhesive strengths of the glue, sodium formate, sodium salicylate (when not in too high concentration and in spite of its great dispersive capacity) and the sugars increase the adhesive strength, and there is a certain amount of evidence that an increase takes place in presence of substances containing hydroxyl-groups.

71. There is no relationship exhibited between the dispersive power of a salt (which is correlated with its alteration of the surface-tension of water) and its effect on adhesive strength; on the other hand the effects of the salts on the jelly strength follow closely their dispersive capacity. If, however, more exact methods can be devised in the future, a study of the action of salts should throw some light on the nature of adhesion and the factors involved in producing a good adhesive.

#### THE CHEMICAL PROPERTIES OF GLUES AND THEIR ADHESIVE STRENGTHS

72. It is obvious that, apart from the fundamental interest of such an inquiry, an examination of the relationship between the chemical properties of glues and their adhesive strengths is

essential if any chemical method of evaluating glues is to be developed. The chemical factors which appear to be most characteristic of the proteins (the group of substances to which gelatin belongs) are the amounts of the different amino-acids which are liberated upon hydrolysis. In other words, in order to characterise a protein accurately, it is necessary to ascertain quantitatively the distribution of the nitrogen between its hydrolytic products. There is no known method by means of which each of the hydrolysis products can be estimated. They can, however, be divided into groups which are dependent on the character of the nitrogen present. The percentages of nitrogen contained in these groups are known as the "Hausmann Numbers." By processes which need not here be described, the "amide" nitrogen, "humin" nitrogen, "diamino" nitrogen, and other nitrogen in the protein are separately estimated, and the amounts of nitrogen in these are calculated as percentages of the total nitrogen in the protein; the figures so obtained are the "Hausmann Numbers" for the protein. It was thought desirable to determine the Hausmann numbers of several glues, and also of a standard gelatin, in order to ascertain whether, in this way, a good glue could be distinguished from a poor one, and also whether the results would indicate the origin of a glue, i.e., whether it had been prepared from bones, cartilage or tendons.

73. The table below gives in a convenient form the results obtained:—

| Sample of Glue.         | Total Nitrogen percentage calculated on dry weight. | Amide Nitrogen. | Humin Nitrogen. | Diamino Nitrogen. | Other Nitrogen. |
|-------------------------|---|-----------------|-----------------|-------------------|-----------------|
| No. 1 Hide              | 16.77   | 1.98            | 0.99            | 25.04             | 71.99           |
| No. 3 Hide              | 17.44   | 2.49            | 0.98            | 21.65             | 74.88           |
| No. 5 Hide              | 17.70   | 2.48            | 1.09            | 22.79             | 73.64           |
| No. 1 Bone              | 17.12   | 3.56            | 1.48            | 21.75             | 73.21           |
| No. 2 Bone              | 16.97   | 3.27            | 0.94            | 21.76             | 74.01           |
| No. 3 Bone              | 17.53   | 2.59            | 0.52            | 21.47             | 75.42           |
| Nelson-Dale Gelatin.    | 16.80   | 2.35            | 0.47            | 19.59             | 77.59           |
| Coignet's "Gold Label." | 17.01   | 1.13            | 0.71            | 20.93             | 77.23           |
| No. A/13                | 17.04   | 3.75            | 0.99            | 18.90             | 76.36           |
| No. B/1                 | 17.07   | 3.32            | 0.58            | 19.63             | 76.47           |
| No. C/1                 | 17.08   | 2.75            | 0.47            | 19.44             | 77.34           |

74. The first six glues were ordinary commercial samples. A/13 was prepared in the laboratory from hard, decalcified bones which were extracted with water for 2-3 hours at 100°C. The glue was dried without clarification or decolourisation.

B/1 was prepared from hard, undecalcified bones by extracting them in an autoclave with water for one hour at 135°C., and afterwards for four hours at 100°C.

C/1 was prepared from cartilage, which had been first treated with 5 per cent. caustic soda, then with dilute hydrochloric acid, and afterwards thoroughly washed. The tissues were then extracted with boiling water.

75. The results indicate that it is not possible to determine the origin of a glue or gelatin by means of the Hausmann numbers. Nevertheless, the differences between the numbers obtained are greater than can be accounted for by experimental errors. They are possibly due to contamination with other proteins or protein decomposition products derived from muscular tissue adhering to the bones, or they may be due to decomposition of skin by the action of lime during treatment in the liming pits.

76. The Hausmann numbers were also determined for gelatins of quite different origins. In the following table the results are given for three samples of such gelatins. No. 1 was described as Brazilian isinglass; No. 2 was similarly described, but was obtained from another dealer; No. 3 was described as Russian isinglass\* :—

| Sample.  | Percentage<br>Nitrogen in<br>dry weight. | Amide<br>Nitrogen. | Humin<br>Nitrogen. | Diamino<br>Nitrogen. | Other<br>Nitrogen. |
|--|--|--------------------|--------------------|----------------------|--------------------|
| No. 1 ...  | 17.28                                    | 3.53               | 0.58               | 20.68                | 75.81              |
| No. 2 ...  | 16.88                                    | 3.26               | 0.35               | 19.52                | 76.87              |
| No. 3 ...  | 17.29                                    | 3.12               | 0.46               | 21.23                | 75.17              |
| Average of the two<br>gelatins in previous<br>table. | 16.9                                     | 1.74               | 0.59               | 20.26                | 77.41              |

77. It will be noticed that the numbers do not differ appreciably from those obtained in the case of mammalian tissues. All the isinglasses, however, differ from the other glues and gelatins in that their solutions on heating to 100° C. yield a precipitate of coagulated albumin. The Russian isinglass contained 4.57 per cent. coagulable protein, whilst Brazilian contained 13.05 per cent.

78. When the Hausmann numbers of the coagulable proteins were determined it was found that the amide-nitrogen was appreciably higher than in the case of ordinary glues and gelatins; they approximate to the "numbers" of egg-albumin, and are given in the following table :—

| Sample of<br>Albumin. | Percentage of<br>Nitrogen in<br>dry weight of<br>sample. | Amide<br>Nitrogen. | Humin<br>Nitrogen. | Diamino<br>Nitrogen. | Monoamino<br>Nitrogen. |
|-----------------------|--|--------------------|--------------------|----------------------|------------------------|
| Brazilian ...         | 14.45  | 6.92               | 2.90               | 20.62                | 69.56                  |
| Russian ...           | 14.67  | 6.20               | 3.06               | 19.43                | 71.31                  |
| Egg ...               | 14.06  | 7.82               | 2.91               | 21.27                | 68.00                  |

\* Isinglass is probably a type of collagen. Brazilian or "Cayenne" isinglass is obtained from a type of cat fish. The Russian variety is made from the "sound" or swimming bladder of several varieties of sturgeon.

79. It is of interest to note that the adhesive strength of the isinglass altered appreciably after removal of the albumins. This is shown by the following table:—

| Sample 1 part in 6 parts water. | Tensile strength in lbs. per sq. inch. |                              |
|---------------------------------|--|------------------------------|
|                                 | Before removal of<br>Albumin.          | After removal of<br>Albumin. |
| Brazilian ...                   | 946                                    | 550                          |
| Russian... ...                  | 1,085                                  | 1,106                        |

80. The Brazilian isinglass, which contains relatively large amounts of coagulable albumin (13·0 per cent) is weakened in adhesive strength after the removal of the impurity.

81. The Hausmann numbers, which can be determined with a fair degree of accuracy, give only a limited amount of information with regard to the chemical composition of a protein or mixture of proteins, and it is very desirable that simple chemical methods should be devised whereby more extensive information as to the amounts of the individual amino-acids can be obtained.

82. An amplification of the Hausmann method has been evolved by van Slyke for the estimation of the amounts of the individual diamino-acids present in the hydrolytic products of proteins by relatively simple methods and a new method, involving an entirely different technique is now being examined by Buston and Schryver (*Biochem. Journ.* 1921, **15**, 636), and it is hoped that it will be possible to apply the procedure to the examination of gelatins and glues, and so to obtain further information as to their variations in chemical composition.

83. Another chemical method has also been applied to the examination of glues. It is possible that, during the extraction of gelatin from its precursors, a certain amount of hydrolysis takes place, especially if traces of acids or alkalis have not been removed before extraction. This should lead to an increase in free amino-groups in the glues.

84. The free amino-groups can be estimated by treatment of a solution of the sample with nitrous acid by the method of van Slyke. The results obtained indicate that there are very few amino-groups in even low grade glues. The differences in the various samples are so small, however, that this method of examination is of little practical value. The results are illustrated in the following table:—

| Samples of glue supplied<br>by the Engineering<br>Standards Association. | Amino-nitrogen in<br>percentage of total<br>Nitrogen of Anhydrous<br>Material. |
|--|--|
| No. 1 ... ... ... ...  | 0·67   |
| No. 3 ... ... ... ...  | 0·64   |
| No. 4 ... ... ... ...  | 0·58   |
| No. 5 ... .. . .   | 0·58   |
| No. 6 ... .. . .   | 0·66   |

85. It further seemed worth while, at this stage, to find out whether there is any connexion between the phosphorus content of a glue and its adhesive strength. Five samples of gelatin glue were examined, the method used being that of Neumann, elaborated by Plimmer. In the table below the glues are arranged in descending order of adhesive strength :—

| Sample of<br>Glue. |     | Phosphorus calculated<br>as percentage of $P_2O_5$ . |
|--------------------|-----|--|
| No. 1              | ... | 0·082  |
| ,, 3               | ... | 0·048  |
| ,, 4               | ... | 0·029  |
| ,, 5               | ... | 0·088  |
| ,, 6               | ... | 0·046  |

86. It will be seen that apparently the amount of phosphorus present in a glue bears no relation to its adhesive strength.

#### STUDY OF THE FORMATION OF GELATIN

87. Glue is an impure gelatin and it is not yet definitely known whether the former owes its superior adhesive power to the presence of some substance or substances other than gelatin. These substances may be degradation products of gelatin, or alternatively, they may be derived from substances quite foreign in nature to gelatin. It should further be noted that the impurities mentioned may not, in themselves, possess high adhesiveness but, by causing a change in the state of aggregation of the gelatin, may indirectly be the cause of the superior adhesive power of the glues containing them.

88. As its chief constituent, gelatin is the primary centre of interest in the investigations of glue which may be analytic or synthetic in character. In an analytical examination of glue, the material would, as far as possible, be separated into its constituents and the physical properties of these would be carefully examined. The most important separation to be undertaken would be that of the gelatin from other nitrogenous constituents. For this purpose it would probably be of advantage to examine the precipitates produced by tannic acid, by trichloracetic acid and by alcohol of gradually increasing strength by which means a partial separation, at least, of the desired nature would be attained. A synthetic examination of glue involves the preparation of various mixtures based on gelatin and an estimation of their value as adhesives. Such a method necessitates the preparation of a standard gelatin, of known history and of the highest purity attainable, and its comparison with a gelatin obtained by the analysis of glue. A synthetic examination of

glue has been undertaken by the Committee. It may be pointed out that commercial gelatins cannot be used in such investigations; their history is unknown and slight variations in the method of manufacture may produce relatively important changes in the composition and properties of the finished product.

89. The Committee consequently considered it an essential preliminary to the study of the factors influencing the quality of a glue, that a standard gelatin should be prepared from a known source by scientifically-controlled methods. Such a study might throw incidental light upon the processes occurring in the manufacture of glue and gelatin; it may be added that this, in fact, has been the case.

90. It would seem that gelatin does not exist, as such, in animal tissues, but is there found in the form of ossein or collagen which are probably anhydrides of gelatin. This view as to the nature of the precursors of gelatin was put forward by Hofmeister more than 40 years ago. Hofmeister found that on heating gelatin at 130°C. for a prolonged period he obtained a substance which could be brought into solution only by continued heating with water, the solution so obtained giving a strong gel on cooling. In the course of the present work Hofmeister's experiment was repeated by heating gelatin *in vacuo* at 140°C. A product similar to his was obtained, and on prolonged heating with water and cooling it yielded a firm jelly. Hofmeister's view is thus partially confirmed. A simple experiment still further strengthens the view. Gelatin is readily dissolved by a normal solution of sodium salicylate in the cold, and a very viscous solution containing 20 per cent. or more of gelatin can thus be prepared. A similar sodium salicylate solution extracts no gelatin from untreated tissues; these must be subjected to the action of water and heat before gelatin is obtained.

91. Several attempts to prepare a standard gelatin have been made by earlier investigators. For example, Berger and Gies, using tendons of Achilles as their raw material, after freeing these from connecting tissue, digested them in cold lime water in order to remove the mucoid substances. The insoluble residue was then decalcified by acetic or hydrochloric acid and finally washed. The authors state that the collagen so prepared was contaminated with a little elastin (a protein derived from connective tissue); a pure product was obtained by digesting the cleaned tendons with trypsin which dissolved all the connective tissue, leaving unattacked the collagenous fibres. The residue was decalcified in the usual way. J. J. Andeer (*Compt. Rend.* 1895, **126**, 1295) states that an aqueous solution of phloroglucinol acts as a powerful decalcifier, but has no action on the most delicate organic tissues; and adds that if, in addition to this reagent, hydrochloric acid is employed as a decalcifier, the resulting ossein contains no calcium salts.

**THE DYNAMICS OF THE FORMATION OF GELATIN FROM ITS  
PRAECURSORS IN ANIMAL TISSUE**

92. *Extraction of Gelatin from Ossein.*—The dynamics of the formation of gelatin from its praecursors have been examined most carefully in the case of decalcified bones. As the reaction involved must take place in heterogeneous solution, it might be supposed that the size of the particles exposed to the action of water would play an important part in determining the rate of reaction. Such, indeed, has been found to be the case, and, as the size can be most conveniently regulated when bone is used, this material was selected for the more complete examination.

93. The results obtained for ossein have already been published (Manning and Schryver, Biochem. Journ. 1921, **15**, 523). It may be mentioned here that in this report "ossein" refers to the material in bones and "collagen" to the gelatin-yielding complex found in tendons and skins.

94. *Preparation of the Materials.*—For the purposes of this research, the material used consisted entirely of the middle portions of the femurs of young oxen (about two years old). These were collected from the slaughter-house as soon as possible after the animals were killed, and their preparation for experiment was commenced immediately after they were brought into the laboratory. They were first washed in running water until the washings were clear, and then sawn into two portions along their length, and the marrow and adhering fatty matter was removed. They were then dehydrated and de-fatted, first by treatment with alcohol and then by ether, and afterwards air-dried. They were again cleaned by hand to remove the last portions of adhering tissues and then crushed in rock-crushers. The crushed particles were then sieved, the largest size used passing through  $\frac{1}{2}$  inch mesh, but not through a  $\frac{3}{16}$  inch mesh; the smallest sized bones used passed a  $\frac{1}{32}$  inch mesh. Altogether five grades of bone were decalcified separately, 3 per cent. cold hydrochloric acid (not above 5°C.) being used for the purpose. The course of the decalcification was followed by the titration of the acid from day to day. This process required from three to seven days, the rate depending on the size of the bone particles. After decalcification, the bones were washed with water till acid-free, then with 1 per cent. ammonia solution, again with water, and finally with alcohol and ether, after which they were dried by a fan.

95. *Method of Experiment.*—The rate of extraction of gelatin from decalcified bones of various graded sizes has been measured at 100° and at 90°C.

96. For the extraction at 100° a weighed quantity of the dried decalcified bones was added to a known volume of boiling water

in a flask fitted with a reflux condenser. The time was taken, the mixture was kept gently boiling and at convenient intervals a small sample of the liquid was withdrawn and filtered through cotton wool. The nitrogen content of the filtered sample was determined by Kjeldahl's method, and the boiling continued until no further increase in nitrogen content occurred. To measure the rate of extraction at 90° the same method was used except that the flask was immersed in a thermostat at the required temperature and its contents kept in motion by a glass stirrer.

97. Fig. 1 shows the results for the extraction of gelatin at 100°. In each case 20 g. of decalcified bones and 300 cc. water were taken. The results are plotted as percentages of the total amount of nitrogen extracted. The total nitrogen extracted varied slightly with different samples of bones, due to differences in degree of dryness. It lay between 0·85 and 0·95 grams per 100 grams solution.

Fig. 2 shows the results of extraction at 90°

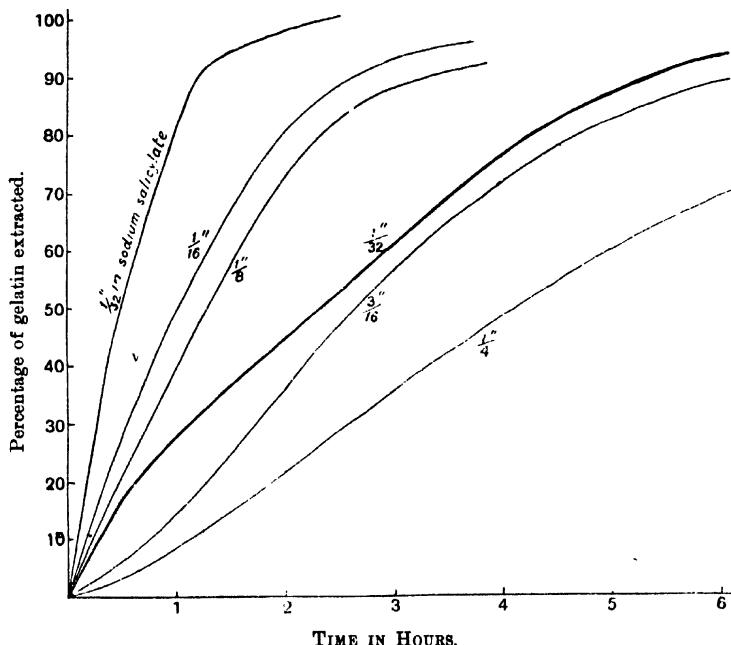


Fig. 1.—Extraction of gelatin at 100°.

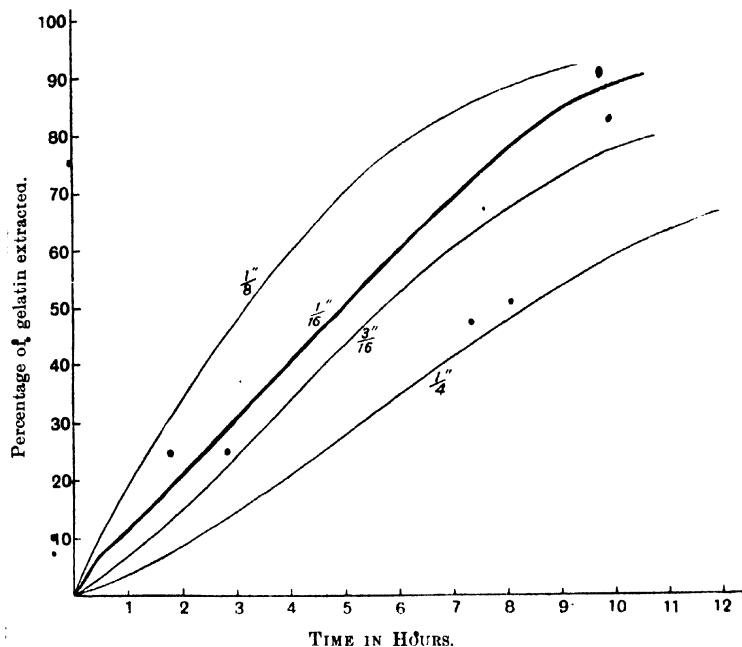


Fig. 2.—Extraction of gelatin at 90°.

98. The extraction curves for the larger sized particles have a point of inflection, and are similar to the curves for autocatalytic reactions. The process of extraction, however, is not autocatalytic. The rate of extraction of a fresh quantity of decalcified bones is unaffected by the presence in solution of some previously extracted gelatin.

99. The rate of extraction increases with the finer division of the bones, except for the very finest ( $\frac{1}{32}$  inch and under). The abnormal curve obtained for the latter size appears to be due to aggregation of the particles. Thus in the presence of sodium salicylate (N/4), a salt which markedly lowers the surface tension of water and which would therefore tend to prevent aggregation, the curve fits normally into the series. A control experiment with  $\frac{1}{16}$  inch bones in presence of N/4 sodium salicylate showed that the salt had no direct effect on the rate of extraction. The reaction rate was slightly greater than in water, but this could be ascribed to the raising of the boiling point of the water. A similar curve was obtained when the reaction with  $\frac{1}{16}$  inch bones was carried out in N/4 sodium chloride solution.

100. It was found that the rate of extraction of gelatin could be expressed by the formula.

$$\frac{1}{a + S_0^2} \log \frac{a(S_0^2 + x)}{S_0^2(a - x)} = kt$$

where :—

$a$  = Total amount of gelatin extractible = 100.

$x$  = Percentage of gelatin extracted in time  $t$ .

$S_0$  = Apparent initial surface (as below).

$k$  = Reaction constant.

101. When "  $k$  " was calculated from the results of experiments carried out at  $90^\circ$  and  $100^\circ\text{C}$ ., the ratio  $\frac{k_{100}}{k_{90}}$  was found to be 2.1.

102. The apparent initial surface was determined by counting the average number of particles contained in one gram of material,  $S_0$  being taken as proportional to  $\sqrt[n]{n}$  where "  $n$  " is the number determined.

103. An attempt was made to measure the surface by estimating the amount of iodine or dyes which could be absorbed by one-gram, size-graded samples of ossein. It was thought that the amount of dye or iodine adsorbed would be proportional to the surface. This was not found to be the case however; a unit weight, whatever the size of the particles, always adsorbed the same amount, although the adsorption equilibrium was reached the more slowly in the case of the larger particles. It is concluded, therefore, that owing to the high porosity of the bones, there is always the same surface available whatever the size of the particles, and that the different rates of extraction from particles of different sizes is controlled largely by the rates of diffusion of gelatin and water through the pores of the ossein; it is essentially a capillary phenomenon.

104. In the equation given above, it will be noticed that as  $S_0$  becomes very large, the reaction becomes unimolecular. Such a reaction would be expected when an anhydride is hydrolysed and it was of interest to investigate the behaviour of gelatin anhydride when hydrolysed by water.

#### DYNAMICS OF THE CONVERSION OF GELATIN ANHYDRIDE INTO GELATIN

105. The anhydride was prepared by the method of Hofmeister. Coignet's gold label gelatin was heated in *vacuo* in an electrically heated furnace at  $140^\circ\text{C}$ . for 8-10 hours. The sheets of anhydride were then allowed to swell in water, and the swollen mass was washed repeatedly, to remove small amounts of decomposition products, and thereafter dried in a current of air. The rates of hydrolysis of this product at  $90^\circ$  and  $100^\circ\text{C}$ . are illustrated by the following curves.

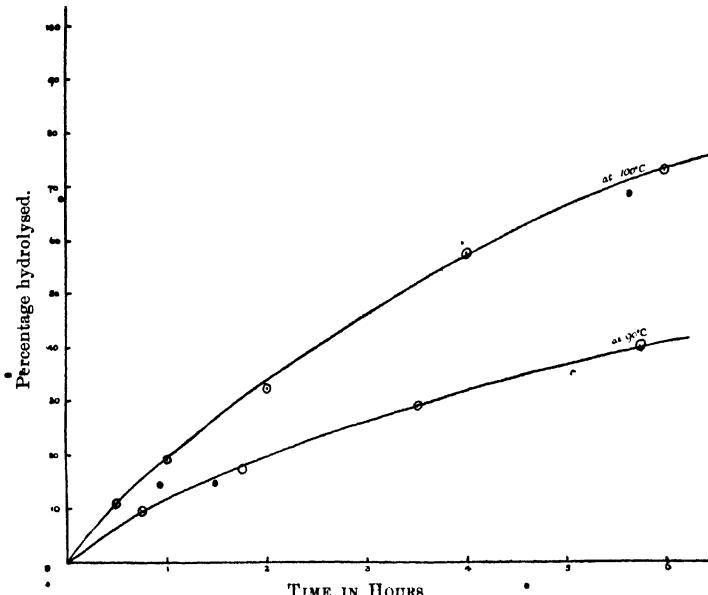


Fig 3.—Hydrolysis of gelatin "anhydride" at 100°C. and 90°C.

106. The results, which indicate an unimolecular reaction, are given in detail below. In each case 20 grms. of gelatin anhydride were heated with 300 c.c. of water.

| Experiment at 100°C.   |                         |                                     | Experiment at 90°C.    |                          |                                     |
|------------------------|-------------------------|-------------------------------------|------------------------|--------------------------|-------------------------------------|
| Time<br>in<br>minutes. | Percentage<br>Extracted | k (for unimole-<br>cular reaction). | Time<br>in<br>minutes. | Percentage<br>extracted. | k (for unimole-<br>cular reaction). |
| 30                     | 10.6                    | 0.097                               | 45                     | 9.9                      | 0.060                               |
| 60                     | 18.9                    | 0.091                               | 105                    | 17.5                     | 0.048                               |
| 120                    | 32.3                    | 0.085                               | 210                    | 29.4                     | 0.043                               |
| 240                    | 57.8                    | 0.094                               | 345                    | 41.0                     | 0.040                               |
| 360                    | 73.6                    | 0.096                               | 435                    | 48.5                     | 0.045                               |
|                        |                         | mean<br>0.0926                      | 680                    | 61.2                     | 0.036                               |

107. The ratio  $\frac{k_{100}}{k_{90}}$  = 2.1, which is exactly the ratio found for the extraction of gelatin from ossein.

108. *Extraction of Gelatin from Tendons.*—Tendons of Achilles were used in these experiments. They were obtained fresh from the slaughter-house, freed from as much connective tissue as possible, and cut into small pieces of approximately equal size; they were then thoroughly washed in repeated changes of water, passed through graded strengths of alcohol—up to absolute—treated with ether and finally air-dried.

109. The curves representing the rate of extraction of gelatin (see above) are precisely similar to those showing the formation

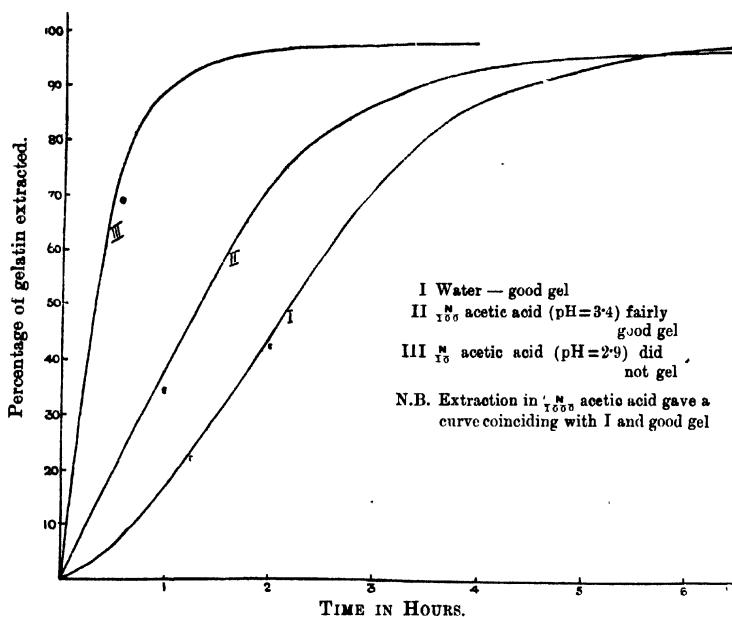


Fig. 4.—Extraction of gelatin from tendons.

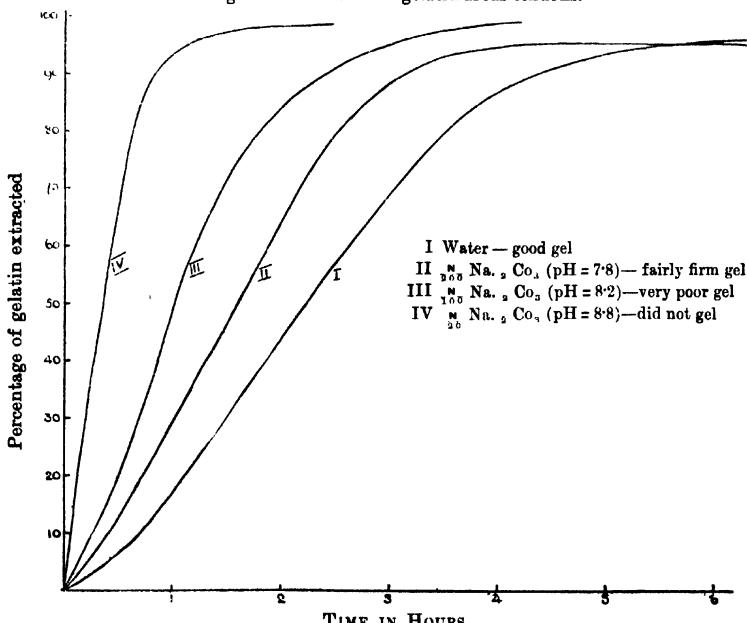


Fig. 5.—Extraction of gelatin from tendons.

of gelatin from ossein. The rate of extraction, once again depends on the size of the particles. Figures 4 and 5 illustrate the reaction.

Average number of particles = 75 in 1 gram whence  
 $S_0 = \sqrt[3]{75} = 4.22$  and  $S^2_0 = 17.8$ .

$$\frac{1}{117.8} \log. \frac{100(17.8 + x)}{17.8(100 - x)} = kt$$

| t (in hours). | x (per cent. extracted). | k       |
|---------------|--------------------------|---------|
| 0.5           | 6.5                      | 0.00279 |
| 1.0           | 17.3                     | 0.00320 |
| 2.0           | 44.2                     | 0.00338 |
| 3.0           | 71.5                     | 0.00352 |
| 4.0           | 87.0                     | 0.00351 |

#### EXTRACTION OF GELATIN FROM SKINS

110. A fresh calf's-skin was freed from hair by scraping (lime is used for this purpose in the manufacturing processes), and cut into small pieces which were then treated in the same way as the tendons.

111. The equation that represents the extraction of gelatin from ossein and collagen, does not hold for the extraction of gelatin from skin—the rate of the latter extraction being more rapid at the start, but slower towards the end; the curve has not yet been mathematically analysed. This divergence may be due either to a chemical difference in the skin collagen or to the different morphological structure (see Figure 6).

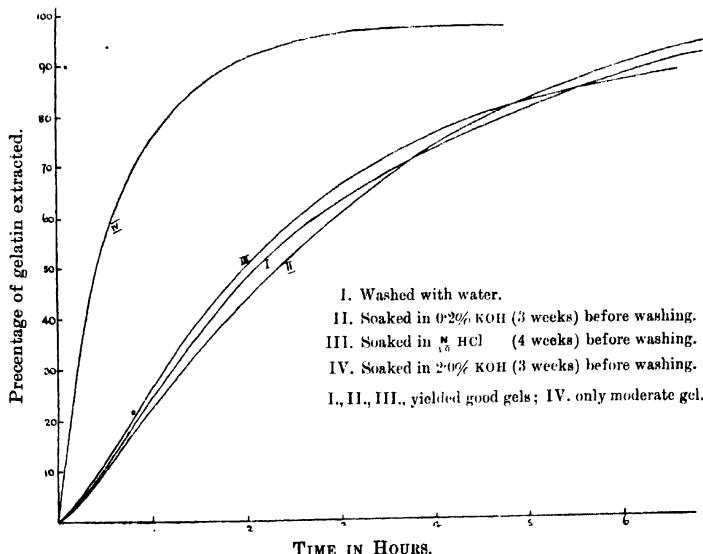


Fig. 6.—Extraction of gelatin from skins.

**112. The action of Alkalies and Acids on the Praecursors of Gelatin.**—In works practice the materials from which glues and gelatins are obtained, are often submitted, if the preliminary operations, to the action of alkalies and acids, as, for example, the liming of skins, the decalcification of bones, and the bleaching of bones or skins by sulphurous acid. It was of importance to determine, therefore, the effect of such reagents on the praecursors of gelatin (*a*) when the acid or alkali was entirely removed before extraction, (*b*) when the reagent was incompletely removed. It should be noted that in order to remove the last traces of the reagents employed in the preliminary treatment, the material, after washing with water until the washings were neutral, was treated appropriately with dilute acid or ammonia. The neutralising agent was then removed by thorough washing, and the material subsequently dried by alcohol and ether as already described.

**113.** Decalcified bones, when treated with 2 per cent. potassium hydroxide for seven days, with 0·2 per cent. potassium hydroxide for fourteen days, or with normal hydrochloric acid for fourteen days gave normal extraction curves and good gels. The ossein was therefore unaffected by the treatment.

**114.** Tendons were unchanged by treatment with normal hydrochloric acid or by 0·2 per cent. potassium hydroxide for fourteen days. Five per cent. potash, however, appeared to have some action in that the gelatin was rapidly extracted, and only a poor gel resulted on cooling.

**115.** The action of alkalies and acids on skin are indicated in the curves in figure 6.

**116.** In conclusion, it may be stated that neither normal hydrochloric acid nor 0·2 per cent. potassium hydroxide has any effect on either tendons, skin or ossein. Two per cent. potassium hydroxide, however, affects both tendons and skin but not decalcified bones. The first two materials when treated with 2 per cent. alkali yield poor gels.

**117.** The tendons contain chondroitin-sulphuric acid. This is extracted by alkalies, and during the course of the above experiments the rate of its extraction was determined by estimating the nitrogen in the alkaline extract from day to day. When 0·2 per cent. alkali was employed, several days elapsed before the nitrogen content reached a maximum; a much shorter period sufficed when 2 per cent. alkali was used.

#### EFFECT OF THE PRESENCE OF ACIDS OR ALKALIS IN THE EXTRACTION FLUID

**118.** It repeatedly happened, in the earlier experiments on the extraction of gelatin from its praecursors, that a rapid extraction took place and a poor gel was obtained. This was found to be due to incomplete removal of acid or alkali; in order

to obtain reliable results the tissue after treatment with alkalis or acids had to be purified as described in the previous section, *i.e.*, washing and neutralising followed by washing and drying.

119. These results indicate that even small amounts of alkali or acid either left in the tissue, or introduced with the extracting fluid, have deleterious effects on the products. It was of interest therefore to determine, in a quantitative manner, the effects of alkalis or acids on the rate of the reaction and on the products formed.

120. The result of the experiments on tendons are indicated by figures 4 and 5. No further remarks on the results, which are evident from the curves, are necessary, beyond the fact that gelatin is more sensitive to alkalis than to acids. If only as little alkali as N/100 sodium carbonate is present a poor gel is obtained, whereas in N/100 acetic acid a fairly good gel was produced.

#### ADDENDUM.—THE EFFECT OF HEAT UPON THE MELTING POINT AND JELLY STRENGTH OF GELATIN

121. In the course of visits paid by the Committee to glue factories, it was observed that in some cases the glue solutions were heated to a high temperature. It was consequently considered of importance to determine the effect, if any, of heat upon the melting point and jelly strength of pure gelatin. For this purpose, 20 per cent. solutions of Congnet's Gold Label gelatin were poured into test-tubes fitted with reflux condensers and heated in an oil bath at (a) 80°C. and (b) 100°C. for various lengths of time. Under these conditions the escape of water from the gelatin solutions during prolonged heating was avoided. The jelly strength was subsequently determined by the method of Cambon and Lipowitz, while the melting point was obtained by the method already described. The results are shown below.

| Time of heating hours. | Melting point.<br>Heated at<br>80°C. | Melting point.<br>Heated at<br>100°C. | Jelly Strength.<br>Heated at<br>80°C. | Jelly Strength<br>Heated at<br>100°C. |
|------------------------|--------------------------------------|---------------------------------------|---------------------------------------|---------------------------------------|
| 0                      | 31.7°C.                              | 31.7°C.                               | 140                                   | 140                                   |
| 1                      | 30.6                                 | 30.0                                  | 100                                   | 70                                    |
| 2                      | 29.8                                 | 29.0                                  | 80                                    | 20                                    |
| 3                      | 29.4                                 | 28.0                                  | 60                                    | nil.                                  |
| 4                      | 29.3                                 | 27.5                                  | 40                                    |                                       |
| 5                      | 28.9                                 | 26.0                                  | 30                                    |                                       |
| 6                      | 28.4                                 | 25.0                                  | 20                                    |                                       |
| 7                      | 27.8                                 | sol.                                  | 10                                    |                                       |
| 8                      | 27.6                                 | sol.                                  | nil.                                  |                                       |
| 9                      | 27.0                                 |                                       |                                       |                                       |
| 10                     | 26.6                                 |                                       |                                       |                                       |
| 11                     | 26.5                                 |                                       |                                       |                                       |
| 12                     | 26.0                                 |                                       |                                       |                                       |
| 24                     | 25.6                                 |                                       |                                       |                                       |
| 48                     | 25.0                                 |                                       |                                       |                                       |
| 96                     | sol.                                 |                                       |                                       |                                       |
| 120                    | sol.                                 |                                       |                                       |                                       |

122. These results show that prolonged heating at these temperatures has a marked deteriorating effect on both the melting point and jelly strength. The latter effect is very notable and would seem to demonstrate the importance of temperature control in the manufacture of glues. It is proposed at a later date to extend these investigations to osmometric measurements for the purpose of determining whether the change during heating is of the nature of degradation of the gelatin.

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### V.—VEGETABLE ADHESIVES

123. The threatened inadequacy of reliable adhesives, in comparison with the requirements of the large aircraft programme of the two closing years of the war, led the original Committee to seek adhesives from new sources. A promising direction in which to look seemed to be towards the vegetable proteins, and especially those which are contained in the residues of seeds used commercially for their oil. Preliminary experiments were therefore made upon the residues remaining after the extraction of the oils from cotton seed, hemp seed, linseed, castor bean and palm kernels. Linseed gave poor results on account of the large amount of mucilage in the seed, while the residues from palm kernels were found to contain only 1 per cent. of protein. Better results were given by cotton and hemp seeds and castor bean residues. Of these the last is most promising; it cannot be used as a foodstuff for cattle since it contains a toxic compound, and hitherto little use has been found for it; to a limited extent it is employed as a fertiliser in the neighbourhood of Marseilles.

124. The castor bean plant (*Ricinus Communis L.*) is indigenous to Africa, but it was early transplanted in India and elsewhere. It is now found in enormous quantities in all tropical and sub-tropical countries, and thus large quantities of the bean were at the time, and are likely to be, available. The following figures furnished by the Board of Trade show the imports into the United Kingdom from 1911 to 1920. The great increase during the latter part of the war is, of course, due to the use of castor oil as a lubricant in aircraft engines.

*Weight and Value of Castor Bean imported into the United Kingdom*

|       | Total Imports. |           | Brazil. |         | British Indu. |           |
|-------|----------------|-----------|---------|---------|---------------|-----------|
|       | Cwts.          | £.        | Cwts.   | £.      | Cwts.         | £.        |
| 1911  | 1,299,147      | 775,532   | 8,191   | 4,882   | 1,288,416     | 769,076   |
| 1912  | 1,095,440      | 667,060   | 1,010   | 538     | 1,086,864     | 661,982   |
| 1913  | 1,205,537      | 710,587   | 487     | 294     | 1,203,355     | 709,061   |
| 1914  | 1,016,690      | 562,081   | 175     | 96      | 1,016,098     | 561,755   |
| 1915  | 556,305        | 359,877   | 120     | 58      | 554,507       | 358,722   |
| 1916  | 819,016        | 733,132   | 3,379   | 3,253   | 813,938       | 728,184   |
| 1917  | 898,557        | 1,281,075 | 11,463  | 13,149  | 886,389       | 1,266,869 |
| 1918  | 1,599,175      | 2,557,401 | 55      | 150     | 1,573,925     | 2,513,078 |
| 1919  | 296,256        | 487,557   | 4,517   | 7,279   | 235,255       | 385,601   |
| *1920 | 292,940        | 440,320   | 93,020  | 147,097 | 124,420       | 177,523   |

\* Provisional.

125. It must be remembered that large quantities of castor bean are used for the production of oil in countries where the castor plant is cultivated.

126. Castor bean meal is rich in protein. The latter is insoluble in water, salt solution and aqueous ammonia, but is soluble in caustic alkalis, sodium carbonate and strong acids. In the present investigation the protein was extracted by digesting the meal at room temperature with 0·4 per cent. sodium hydroxide solution for 3 to 4 hours. After filtration and clearing in a Sharples' centrifuge, the solution was neutralised by the addition of hydrochloric acid. The precipitated protein was then thoroughly washed and dried by one of the following three methods :—

- (a) ether treatment;
- (b) exposure to a current of air;
- (c) scale drying after solution in the minimum quantity of alkali.

127. Attempts made to substitute sodium carbonate or acids for the sodium hydroxide in the extraction process were ineffective.

128. The material thus obtained by alkali extraction is doubtless a mixture of proteins. It cannot be used directly as an adhesive, but must be mixed with a base and a salt. Of the bases tried, magnesia together with caustic soda gave good results, but zinc oxide and barium hydroxide were found to be unsatisfactory. Slaked lime was, on the whole, found to be the most convenient base, and was generally employed. A number of experiments were carried out to discover the most suitable salt and to determine the optimum relative proportions of protein, salt, alkali and water.

129. For the preparation of the mixtures the dry and finely-powdered protein, salt and alkali were thoroughly mixed and added to water in a mortar, with careful stirring; a stiff jelly is thus formed in a few minutes. On warming the jelly to 60° C. it becomes a thick, sticky liquid, which is then ready for use as an adhesive. Alternatively, the jelly is allowed to stand for a considerable time, when in most cases it liquefies spontaneously. The liquefaction is probably due to the breaking down of the protein by the alkali, a process which is accelerated by heat.

130. In a series of experiments designed to ascertain the most suitable salt to mix with the protein and alkali, the following proportions were used :—

|             |     |       |  |
|-------------|-----|-------|--|
| Protein     | ... | ...   | 3 parts by weight.                             |
| Slaked lime | ... | 1     | " "  |
| Alkali salt | ... | 0·6—1 | " "  |
|             |     |       | according to the molecular weight of the salt. |
| Water       | ... | ...   | 8 parts by weight.                             |

131. The results obtained with the different mixtures are tabulated below :—

| Salt used.  | Resulting Mixture.                             | Effect of heating.                                |
|---|--|---|
| Sodium Fluoride ... ...                                 | Stiff jelly ...                                | Melts sticky.                                     |
| Sodium Chloride ... ...                                 | Paste ... ...                                  | Does not liquefy.                                 |
| Sodium Arsenate ... ...                                 | Stiff jelly ...                                | Does not liquefy.                                 |
| Disodium Hydrogen Phosphate                             | Jelly ... ...                                  | Melts.  |
| Sodium Thiosulphate ... ...                             | Paste ... ...                                  | Does not liquefy.                                 |
| Sodium Acetate ... ...                                  | Paste ... ...                                  | Does not liquefy.                                 |
| Sodium Salicylate ... ...                               | Stiff jelly paste ...                          | Liquefies, sticky.                                |
| Potassium Nitrate ... ...                               | Stiff paste ...                                | Hardens.  |
| Sodium Potassium Tartrate ...                           | Stiff jelly ...                                | Liquefies, sticky.                                |
| Sodium Sulphite... ...                                  | ... ...  | Liquefies, sticky.                                |
| Sodium Sulphate ... ...                                 | Paste ... ...                                  | Dries.  |
| Potassium Ferricyanide ...                              | Paste ... ...                                  | Dries.  |
| Sodium Silicate ... ...                                 | Jelly ...                                      | Liquefies, sticky.                                |
| Sodium Hyposulphite ... ...                             | Stiff paste ...                                | Does not liquefy.                                 |
| Sodium Nitrite ... ...                                  | Stiff jelly paste ...                          | Does not liquefy.                                 |
| Potash Alum ... ...                                     | Stiff paste ...                                | Does not liquefy.                                 |
| Sodium Naphthalene Sulphonate                           | Stiff jelly ...                                | Does not liquefy.                                 |
| Sodium Naphthalene Sulphonate<br>(in excess with lime). | ... ... ...                                    | Liquefies not readily :<br>lumpy.                 |
| Sodium Benzene Sulphonate ...                           | Same as sodium<br>naphthalene sul-<br>phonate. |   |
| Borax ... ... ... ...                                   | Paste ... ...                                  | Does not liquefy.                                 |
| Potassium dihydrogen Phos-<br>phate.                    | Stiff jelly paste ...                          | Does not liquefy.                                 |
| Potassium dihydrogen Phos-<br>phate (with excess lime). | ... ... ...                                    | Liquefies.  |
| Sodium Stannate ... ...                                 | Stiff jelly ...                                | Liquefies, sticky.                                |
| Sodium Silicofluoride ... ...                           | Stiff jelly ...                                | Liquefies, sticky.                                |
| Sodium Citrate ... ... ...                              | Jelly ... ...                                  | Liquefies, sticky.                                |
| Ammonium Phosphate ... ...                              | Stiff paste ...                                | Does not liquefy.                                 |
| Ammonium Phosphate (in ex-<br>cess).                    | ... ... ...                                    | Liquefies with diffi-<br>culty.                   |
| Ammonium Fluoride ... ...                               | Paste ... ...                                  | Behaves as in case of<br>Ammonium Phos-<br>phate. |

132. From the results obtained it was clear that sodium fluoride is the most satisfactory salt to employ.

133. It was next necessary to ascertain the proportions in which the protein, salt, lime and water should be mixed to produce a cement giving in a joint the greatest possible adhesive strength. It should here be noted that if insufficient alkali be added the breakdown of the protein does not proceed sufficiently far for the formation of an adhesive ; if, on the contrary, too much is added, the breakdown is too far-reaching for a good adhesive to be formed. The amount of water used is also an important factor ; the proportions of the several constituents here noted were only ascertained after some hundreds of mixtures had been prepared and several times as many joints had been made and tested. The strength tests were made with American Walnut joints in the

manner already described; three or four joints were made with each mixture, and the mean tensile strength of these was determined.

134. In the following table the results of a series of tests with sodium fluoride mixtures are given; the table serves to indicate the manner in which this part of the investigation was approached:—

TABLE C.

| Series. | Protein. | Sodium Fluoride. | Slaked lime. | Water.    | Mean Strength of joint.<br>(lbs./sq. in.) |
|---------|----------|------------------|--------------|-----------|---|
| A. 1    | 6 parts. | 0·6 parts.       | 2 parts.     | 20 parts. | 478                                       |
| A. 2    | 6 "      | 0·6 "            | 1·5 "        | 20 "      | 1,008                                     |
| A. 3    | 6 "      | 0·6 "            | 1 "          | 19 "      | 1,016                                     |
| B. 1    | 6 "      | 1·2 "            | 2 "          | 23 "      | 1,084                                     |
| B. 2    | 6 "      | 1·2 "            | 1·5 "        | 20 "      | 1,036                                     |
| B. 3    | 6 "      | 1·2 "            | 1 "          | 16 "      | No joints. Too thick to be practicable.   |
| C. 1    | 6 "      | 1·5 "            | 2 "          | 14 "      | 745                                       |
| C. 2    | 6 "      | 1·5 "            | 1·5 "        | 14 "      | 627                                       |
| C. 3    | 6 "      | 1·5 "            | 1 "          | 14 "      | No joints, does not liquefy readily.      |
| D. 1    | 6 "      | 2 "              | 2 "          | 15 "      | 622                                       |
| D. 2    | 6 "      | 2 "              | 1·5 "        | 14 "      | 669                                       |
| D. 3    | 6 "      | 2 "              | 1 "          | 14 "      | Did not liquefy.                          |
| E. 1    | 6 "      | 0·6 "            | 2 "          | 17 "      | 605                                       |
| E. 2    | 6 "      | 0·6 "            | 1·5 "        | 16 "      | 857                                       |
| F. 1    | 6 "      | 1·2 "            | 2 "          | 16 "      | 944                                       |
| F. 2    | 6 "      | 1·2 "            | 1·5 "        | 15 "      | 734                                       |
| G. 1    | 6 "      | 1·2 "            | 2·5 "        | 25 "      | 1,042                                     |
| G. 2    | 6 "      | 1·2 "            | 2 "          | 27·5 "    | 913                                       |
| G. 3    | 6 "      | 1·2 "            | 2·3 "        | 21 "      | 1,008                                     |
| H. 1    | 6 "      | 0·9 "            | 1·5 "        | 21 "      | 1,034                                     |
| H. 2    | 6 "      | 1 "              | 1·76 "       | 22 "      | 711                                       |
| H. 3    | 6 "      | 1·1 "            | 1·94 "       | 22·5 "    | 893                                       |
| H. 4    | 6 "      | 1·2 "            | 2·1 "        | 23 "      | 745                                       |
| H. 5    | 5·5 "    | 1·2 "            | 2·1 "        | 22 "      | 705                                       |
| H. 6    | 5 "      | 1·2 "            | 2·1 "        | 21 "      | 563                                       |
| I. 1    | 6 "      | 1·2 "            | 2 "          | 23 "      | 975                                       |
| I. 2    | 6 "      | 1·2 "            | 2 "          | 20·5 "    | 980                                       |
| I. 3    | 6 "      | 1·2 "            | 2 "          | 18·5 "    | 907                                       |
| I. 4    | 6 "      | 1·2 "            | 2 "          | 16 "      | 747                                       |
| I. 5    | 6 "      | 1·2 "            | 2 "          | 14 "      | 487                                       |
| I. 6    | 6 "      | 1·2 "            | 2 "          | 25 "      | 913                                       |
| J. 1    | 7·5 "    | 1 "              | 1·5 "        | 25 "      | 940                                       |
| J. 2    | 7·5 "    | 1 "              | 1·5 "        | 22·5 "    | 840                                       |
| J. 3    | 7·5 "    | 1 "              | 1·5 "        | 27·5 "    | 995                                       |

135. From the above results it will be seen that of the sodium fluoride cements, B.1, if used immediately after preparation, appears to be the most satisfactory as regards tensile strength. Unfortunately, all these alkaline cements rapidly diminish in strength on keeping. For example, the initial tensile strength

of the best of the cements (B.1) fell from 1,084 to 956 lbs. per square inch in two hours; indeed on long standing it became thin and useless as a cement, doubtless owing to the continued action of the alkali upon the protein.

136. A cement which exhibited better keeping qualities was found to consist of protein (40 parts), sodium carbonate (8 parts), slaked lime (10 parts), and water (160 parts). The initial tensile strength of this cement was 840 lbs. per square inch, the value falling to 640 lbs. on standing for 20 hours.

137. The diminution in tensile strength on standing being probably due to the influence of the alkali, it was thought that by neutralising the alkalinity after the mixture had passed into solution an adhesive of improved keeping quality might be obtained. In further experiments therefore, the alkalinity of the mixture was reduced as far as possible without causing precipitation of the protein, by the addition of various organic acids. This procedure certainly had the effect of prolonging the effective life of the liquid adhesive, but the tensile strength was much lowered. Thus an adhesive to which formic acid had been added in this way exhibited a tensile strength of only 680 lbs. falling to 640 lbs. per square inch in the course of 24 hours.

138. During these experiments it was found that if a cement solution of the character of B.1 above be prepared from protein, lime and sodium fluoride, and the protein precipitated from this by the addition of acid, the latter had its properties modified by the treatment. It is possible to prepare from this altered protein an adhesive containing less alkali than has hitherto been found necessary. As was to be expected, this new adhesive did not deteriorate rapidly on keeping. A mixture was made of

|                  |     |     |    |      |       |
|------------------|-----|-----|----|------|-------|
| Protein          | ... | ... | .. | 78   | grams |
| Sodium carbonate | ..  | ... | .. | 4·5  | "     |
| Sodium fluoride  | ... | ..  | .. | 4·0  | "     |
| Slaked lime      | ... | ..  | .. | 12·5 | "     |
| Sodium arsenate  | ..  | ... | .. | 1·0  | "     |
| Water            | ... | ..  | .. | 250  | c.c.  |

and was found to have a tensile strength of 547 lbs. per square inch. From this mixture the protein was precipitated by the addition of acid and was used in preparing a fresh adhesive of similar composition to the above mixture. This adhesive possessed an initial tensile strength of 1,100 lbs. falling to 1,005 lbs. per square inch on standing for four hours.

139. The change in the protein which is brought about as described above might be imitated, it was thought, by digestion with caustic soda. Many experiments were undertaken with this object in view, but they were uniformly unsuccessful, the resulting products being unsatisfactory.

140. As the outcome of these investigations, several formulae for castor bean protein cements have been evolved; among them may be mentioned the following :—

|       |                  |     |     |     |     |     | Farts. |
|-------|------------------|-----|-----|-----|-----|-----|--------|
| (i)   | Protein          | ... | ... | ... | ... | ... | 78     |
|       | Sodium carbonate | ... | ... | ... | ... | ... | 45     |
|       | Sodium fluoride  | ... | ... | ... | ... | ... | 4      |
|       | Sodium arsenate  | ... | ... | ... | ... | ... | 1      |
|       | Slaked lime      | ... | ... | ... | ... | ... | 12.5   |
|       | Water            | ... | ... | ... | ... | ... | 200    |
| (ii)  | Protein          | ... | ... | ... | ... | ... | 60     |
|       | Sodium fluoride  | ... | ... | ... | ... | ... | 6      |
|       | Slaked lime      | ... | ... | ... | ... | ... | 10     |
|       | Water            | ... | ... | ... | ... | ... | 190    |
| (iii) | Protein          | ... | ... | ... | ... | ... | 75     |
|       | Sodium carbonate | ... | ... | ... | ... | ... | 10     |
|       | Slaked lime      | ... | ... | ... | ... | ... | 1      |
|       | Water            | ... | ... | ... | ... | ... | 250    |

141. Experience with these adhesives seemed to show that they might possibly prove suitable for the production of plywood. A sample of the latter was accordingly made with a cement prepared according to the following formula :—

78 parts protein (reprecipitated after fluoride lime treatment).  
 4·5 „ sodium carbonate.  
 4 „ sodium fluoride.  
 1 „ sodium arsenate.  
 12·5 „ slaked lime.  
 250 „ water.

142. Tests of this wood were made in the laboratories of the Aircraft Inspection Dept. in accordance with the Air Board Specification, and the following tensile strengths were obtained :—

|             |       |      |     |     |       |
|-------------|-------|------|-----|-----|-------|
|             | 198   | lbs. | per | sq. | inch. |
| 216         | "     | "    | "   | "   | "     |
| 165         | "     | "    | "   | "   | "     |
| 162         | "     | "    | "   | "   | "     |
| 150         | "     | "    | "   | "   | "     |
| 180         | "     | "    | "   | "   | "     |
| Average ... | 178·5 | "    | "   | "   | "     |

143. It will be seen that the average result is well above the standard figure—150 lbs./sq. in.—for plywood, while no individual result falls below it.

144. These investigations have shown that it is possible to utilise the proteins of the castor bean in the production of a satisfactory substitute for casein cements. Unfortunately, however, it is necessary to subject the proteins to a preparatory treatment—precipitation from an alkali fluoride mixture—in order to obtain a cement which does not deteriorate rapidly on standing. This treatment makes it impossible to produce the protein cement at a cost which would enable it to compete with casein adhesives in the open market.

## VI.—OTHER ADHESIVES

145. In addition to the researches already described, the Committee have made further exploratory enquiries concerning other adhesives. While these enquiries have not led to any very positive or useful results, it may be advantageous briefly to refer to them here.

146. Blood adhesives, which contain the coagulable protein of blood, are put to an important use in the manufacture of plywood. Experimental work in connexion with these adhesives was commenced by the original Committee, but it was relinquished during the war in favour of more important or more promising lines of work; the results were not of sufficient significance for description in this report.

### PHENOL-FORMALDEHYDE CONDENSATION PRODUCTS

147. Much attention has been given during recent years to the products of the phenol-formaldehyde condensation, but this attention has been very largely directed to the production of plastic materials rather than of adhesives. Many references to these materials are to be found in the technical literature which it is impossible to review here.

148. It may be recalled, however, that perhaps one of the most successful of the earlier methods of producing these condensation products was that of Story (Brit. Pat. 8875/1905), who used excess of phenol, and obtained a viscous mass which was poured into moulds and slowly dried at 80-100°C. The product was an infusible, insoluble, transparent mass which could easily be worked. Baekeland (*see Journ. Ind. Eng. Chem.* 1909, I, 545) modified this process by condensing equimolecular quantities of phenol and formaldehyde in the presence of a small quantity of a base. The reaction proceeds in two stages, viz. :—

- (i) condensation of phenol and formaldehyde, in the presence of a catalyst, with the formation of a liquid or viscous product soluble in alcohol, glycerine, acetone, etc. ;
- (ii) transformation of the first product into an insoluble and infusible mass by heating it at high temperatures under pressure.

The final product is the material known as Bakelite. Subsequently Baekeland described (Brit. Pat. 26614/1911) an improved cardboard, in which was used as binding agent the product obtained in the first stage of the reaction, dissolved in alcohol. In view of this use of the condensation products, it appeared possible that an adhesive of this nature might be used in the manufacture of plywood.

149. To test this possibility two panels of plywood were prepared, using as a binding agent an alcoholic solution of the first condensation product as described under (i) above. The results were encouraging, but certain difficulties became evident in the

course of the work. The solvent alcohol is expensive, and it is impossible to avoid its rapid evaporation since the solution must be applied hot; the result is that an uneven layer of adhesive is obtained. Different portions of the two plywoods prepared gave irregular results on testing, a fact which points to unevenness in the application of the adhesive; thus the tensile strength of a number of samples of the two panels were found to vary between 136 and 380 lbs. per square inch.

A further difficulty arises from the fact that in order to convert the soluble condensation product into the insoluble and infusible one, it is necessary to heat the wood to temperatures at which charring begins. The plywood so obtained was, in consequence, not so elastic or flexible as the ordinary plywood made up with blood adhesives.

150. When, instead of using equimolecular proportions of phenol and formaldehyde, excess of the former was employed, the first product was transformed into an insoluble second product at a lower temperature; moreover, the end product was more elastic than before, and could be used in admixture with camphor or even gelatin. The adhesive was prepared as follows:—100 parts of phenol, commercial carbolic acid or mixed cresols were heated on a water-bath, under a reflux condenser, with 50 to 80 parts of 40 per cent. commercial formaldehyde solution. When pure phenol or cresols were used it was found necessary to employ a small quantity of a catalyst in the form of a mineral or an organic acid, a mineral or an organic base, or a salt which yields a weak acid and a strong base on hydrolysis. If the catalyst was used in any but small quantity the reaction was difficult to control. The heating was continued for 8-10 hours. When the highest proportion of formaldehyde was used (80 parts) the reaction mixture separated into two layers the upper of which could be removed; this, however, was not essential. The product was a liquid either transparent or of creamy appearance, according to the nature of the phenols employed. The liquid was evaporated down in open vessels to the consistency of a thick syrup; an excess of ammonia solution was then added, and the heating continued for a short time to drive off the bulk of the ammonia.

151. The adhesive so obtained has the consistency of ordinary glue solution at the same temperature, and can be applied by brush or by heated rollers. On cooling it turns to a thick jelly and can be stored in this form; it is not attacked by bacteria. As indicated above, this material does not set at ordinary temperatures, and it is necessary to apply heat in order to transform it into the infusible, insoluble material which is the actual adhesive. This heating can be effected by the ordinary processes used in the manufacture of plywood with blood cements.

152. In mechanical trials of this adhesive, the fracture in every case occurred in the wood at pulls of between 1,288 and 896 lbs. per square inch; it was, therefore, impossible to compare the

results with those given by ordinary glues, but it is probable that the average strength is at least equal to that of good airscrew glue. Plywood prepared with wet veneers had good waterproof qualities, and the shear test gave an average value of 212 lbs. per square inch, which is little above the value for ordinary veneers. With dry veneers the results were much higher; panels prepared with the condensation product were compared with plywood made from several casein and blood cements, the comparison being generally favourable to the condensation product.

153. These various plywoods were submitted to further tests to ascertain the resistance of the cement to moisture and heat. An immersion test indicated that the phenol-formaldehyde adhesive may be considered absolutely waterproof. In a prolonged weathering test panels prepared by means of the condensation product remained intact while all other panels failed. Finally, heat tests further revealed the superiority of the phenol-formaldehyde cement as compared with other adhesives used in plywood making. •

154. It may be noted that while the above tests were carried out on plywood made from birch and poplar, the phenol-formaldehyde product can be used with other woods. It possesses the additional advantages that it does not affect the colour of mahogany and similar woods (as do casein and blood cements), and it does not alter the colour of stained veneers.

155. In concluding this section of the report it may be mentioned that the work upon the phenol-formaldehyde adhesives was carried out entirely during the war period, when it appeared to have an important significance. With the end of the war its importance for the Fighting Services diminished, while its industrial development was found to be already covered by British Patent 129993/1919. All work upon the adhesive was, therefore, stopped shortly after the end of the war.

